FORMULATION AND *IN-VITRO* EVALUATION OF METOPROLOL TARTRATE MICROSPHERES USING NATURAL, SEMI SYNTHETIC AND SYNTHETIC POLYMER AS CONTROLLED RELEASE DOSAGE FORM

A Dissertation Submitted to

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In partial fulfillment for the award of Degree of

MASTER OF PHARMACY

(Pharmaceutics)

Submitted by

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MELMARUVATHUR - 603 319

APRIL 2013

CERTIFICATE

This is to certify that the research work entitled "**FORMULATION AND *IN-VITRO* EVALUATION OF METOPROLOL TARTRATE MICROSPHERES BY USING NATURAL SEMISYNTHETIC AND SYNTHETIC POLYMERS AS CONTROLLED RELEASE DOSAGE FORM"** submitted to The Tamil Nadu Dr.M.G.R. Medical University, Chennai in partial fulfillment for the award of the Degree of the Master of Pharmacy (Pharmaceutics) was carried out by **"CHUKKAPALLI SAMATHA" (Register No. 26116006)** in the Department of Pharmaceutics under my direct guidance and supervision during the academic year 2012-2013.

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Dedicated

To

My beloved parents...

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CHUKKAPALLI SAMATHA

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ABBREVIATIONS

%	----	Percentage
<	----	Less Than
>	----	More Than
°C	----	Degree Celsius
µg	----	Microgram
cm	----	Centimeter
CDDS	----	Controlled drug delivery system
DE	----	Dissolution Efficiency
DSC	----	Differential Scanning Calorimetry
F	----	Formulation
FTIR	----	Fourier Transform-Infra Red Spectroscopy
gm	----	Grams
HCl	----	Hydrochloric acid
hrs	----	Hours
ICH	----	International Conference on Harmonization
IP	----	Indian Pharmacopoeia
MDT	----	Mean Dissolution Time
mg	----	Milligram
ml	----	Milli liter
mm	----	Millimeter
N	----	Normality
nm	----	Nanometer

PBS	----	Phosphate Buffer Solution
PH	----	Pulmonary hypertension
RH	----	Relative Humidity
Rpm	----	Revolutions per Minute
S. No.	----	Serial Number
SEM	----	Scanning electron microscope
T	----	Time
USP	----	United State Pharmacopoeia
UV	----	Ultra Violet
W/v	----	weight/volume
W	----	weight
λ max	----	Absorption maximum

INTRODUCTION...

1. INTRODUCTION

1.1. NOVEL DRUG DELIVERY SYSTEM *(Bankar G.S and Rhodes C.T., 2009; Brahmankar D.M and Jaiswal S.B., 2005; Chein Y.W., 2002)*

The goal of a controlled release dosage form is to maintain therapeutic blood or tissue levels of the drug for an extended and specified period of time. This is generally accomplished by attempting to obtain "zero-order" release from the dosage form. Zero-order release constitutes drug release from the dosage form which is independent of the amount of drug in the delivery system (i.e. a constant release rate). Sustained-release systems generally do not attain this type of release and usually try to mimic zero-order release by providing drug in a slow first-order fashion (i.e., concentration release dependent). Systems that are designated as prolonged release can also be considered as attempts at achieving sustained-release delivery.

The term "Controlled- release drug product" has been used to describe various types of oral extended release rate dosage forms, including sustained release (SR), sustained action, prolonged action, long action and retarded release. These terms for extended release dosage forms were introduced by drug companies to reflect a special design for producing an extended release (ER) dosage form or used as a marketing term.

In the last two-three decades interest in sustained release drug delivery systems is remarkably increasing. This has been due to various factors viz.

- Developing new drug entities.
- Expiration of international patents
- Discovery of new polymeric materials suitable for prolonging the drug release.

- Need of therapeutic efficacy and safety achieved by sustained release drug delivery.

The subject of controlled release has been reviewed by various authors. Several books have been published on it. These reviews and books provide not only the mechanisms and technology of production of dosage forms but also the information on clinical evidence and performance.

There are many definitions of controlled release but the simplest definition is "Any drug or dosage form or medication that prolongs the therapeutic activity of drug''. The overall objective is that, once the drug-carrier material has been injected or otherwise implanted or taken orally into the body, the drug is released at a predetermined rate for some desired period of time. Controlled release technology is relatively new field and as a consequence, research in this field has been extremely fertile and has produced many discoveries.

Non-immediate release delivery systems may be divided conveniently into 4 categories,

A. Delayed release

B. Sustained release

a) Controlled release

b) Prolonged release

C. Site- specific release

D. Receptor release

Delayed – release systems are those that use repetitive, intermittence dosing of a drug from one or more immediate release units incorporated into a single dosage forms to make delayed action. Example: Repeat- action tablets and capsules, enteric coated tablets where timed release achieved by a barrier coating.

Sustained- release systems includes any drug delivery system that achieves slow release of drug over an extended period of time.

Controlled release systems are those systems which are successful maintaining constant drug levels in blood or target release (i.e.) release rate of drug occurs in controlled manner.

Prolonged released systems only extends the duration of action and drug release that achieved by conventional drug delivery.

Site specific and receptor release refers to targeting of drug directly to a certain biological location. In the case of site- specific release, the target is a certain organ or tissue, for receptor release, the target is the particular receptor for a drug within an organ or tissue.

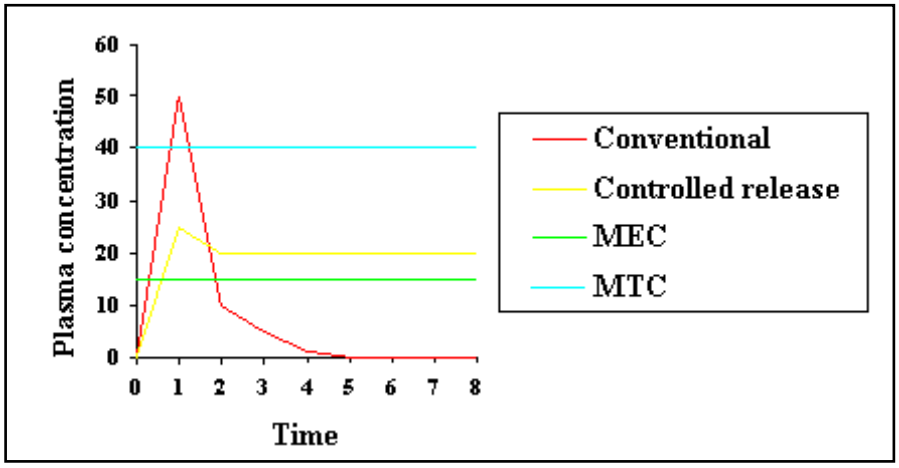

Fig: 1.1**:** Controlled drug release profile

1.1.1. Advantages of Novel Drug Delivery Systems:

(Shoba Rani R Hire math., 2008)

- Improved therapy by increasing the duration of action and reduced side effects.
- Increase the patient compliance through decreased dosing frequency and convenient routs of administration.

- Achieve targeting of drugs to a specific site to reduce unwanted side effects.

1.2. CONTROLLED DRUG DELIVERY:

One of essential issues of drug formulation is the controlled release of drugs, which can improve therapeutic efficacy by offering prolonged in vivo action, controlled blood concentration as well as tissue-targeted local release. A possible approach to the biodegradable polymer microspheres. Controlled drug delivery occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or other active agent in such a way that the active agent is released from the material in a predesigned manner. The release of the active agent may be constant or cyclic over a long period, or it may be triggered by the environment or other external events. In any case, the purpose behind controlling the drug delivery is to achieve more effective therapies while eliminating the potential for both under- and overdosing. The release patterns of drug from both formulations traditional and controlled are given in fig 1.2.

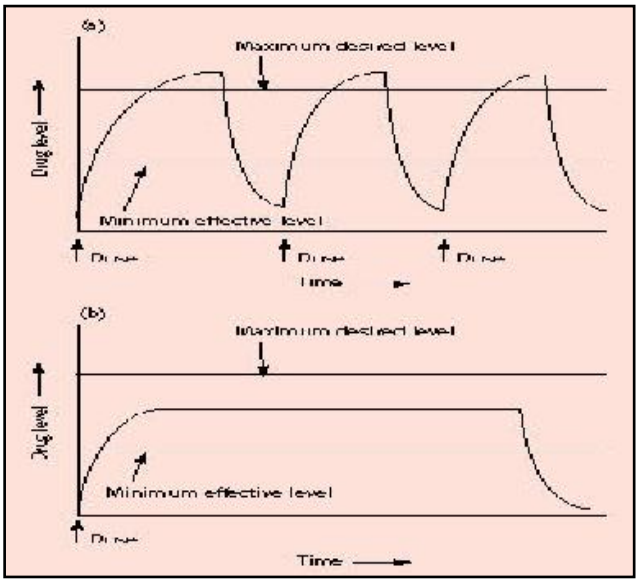

Fig: 1.2: Drug levels in the blood with (a) traditional drug dosing and (b) controlled- Delivery dosing.

Definition for controlled drug delivery system:

Controlled release refers to the use of a delivery device with the objective of releasing the drug into the patient body at a predetermined rate, or at specific time or with specific release profiles. This could revolutionize the manner of medication effects and offer following advantages along with some disadvantages.

Advantages of control release dosage form:

- Reducing the dosing frequency.
- Reduced fluctuations in circulatory drug levels.
- Avoidance of patient compliance.
- Decreased side effects like reduced GI irritation.

Disadvantages of control release dosage form:

- High cost
- Un predictable or poor in vivo-In-vitro correlation
- Dose dumping
- Reduced potential for dose adjustment
- Poor systemic availability
- Increased first pass clearance.

Various characteristics of drug molecule that render it unsuitable for controlled release dosing :

- Narrow therapeutic index
- Short/long elimination half life
- Poor absorption

- Active absorption large doses
- Low aqueous solubility
- Extensive first pass metabolism
- Incompatible pharmacological and
- Circulation time course

Biopharmaceutical aspects of regulatory requirement and new drug applications:

The controlled release formulation developed should aim to accomplish two important Objectives:

- It should allow a maximum possible percentage of the dose in the formulation to be absorbed in controlled manner.
- It should be capable of minimizing patient-to-patient variability.

Over the past three decades, considerable research interest has arisen worldwide in the development of new colloidal drug delivery systems. The ideal colloidal delivery system could transport the associated drug to its desired site of action and then release it at an optimum rate. The carrier itself should be non-toxic and able to be degraded in vivo so that it does not accumulate indefinitely in the tissues. The colloidal preparation also needs to be pharmaceutically acceptable with regards to stability and ease of administration.

PREREQUISITES FOR A DRUG TO BE SUITABLE FOR DESIGN OF ORAL CONTROLLED RELEASE DOSAGE FORM *(Bandyopadhyay A .K., 2008)*

Some characteristics make a drug more suitable for extended release dosing, such as

1. Elimination half-life between 2 to 8 hours.
2. Broader therapeutic index.
3. Moderate unit dose.
4. Significant extent of absorption in GIT.
5. Optimum solubility characteristics.
6. Minimal first-pass clearance

FACTORS INFLUENCING THE DESIGN AND PERFORMANCE OF CONTROLLED RELEASE PRODUCTS:

Oral drug delivery is the most widely utilized route of administration among all the routes that have been explored for the systemic delivery of drugs via various pharmaceutical products in different dosage forms. Irrespective of their mode of delivery (immediate, sustained or controlled release) and the design of dosage forms (either solid or liquid) they must be developed within the intrinsic characteristics of GIT physiology. Therefore a fundamental understanding of pharmacokinetics, pharmacodynamics and formulation design is essential to achieve a systematic approach to the successful development of an oral pharmaceutical dosage form. A number of variables such as drug properties, route of delivery, target sites, duration of therapy, the disease state and patient variables must be considered. The formulation and performance of sustained release products are greatly influenced by the physicochemical and biological properties of drug.

1.3. PRINCIPLE BEHIND SR/CR DRUG RELEASE: *(Bankar G.S and Rhodes C.T., 2009; Brahmankar D.M and Jaiswal S.B., 2009; Robinson J.R and Lee V.H.L., 2005)*

Dissolution and diffusion controlled systems have classically been of primary importance in oral delivery of medication because of their relative ease of production and cost compared with other methods of sustained or controlled delivery. Most of these systems are solids, although a few liquids and suspension have been recently introduced.

The classification of such systems is as follows:

1. Diffusion controlled systems.
2. Dissolution controlled systems.
3. Dissolution and Diffusion controlled systems.
4. Osmotically controlled systems.
5. Ion exchange systems.

1. Diffusion Controlled Systems

Diffusion systems are characterized by the release rate being dependent on its diffusion through an inert membrane barrier. Usually this barrier is an insoluble polymer. In general two types of sub classes of diffusion systems are recognized they are

a. Reservoir devices.
b. Matrix devices.

a. Reservoir devices

Reservoir devices are characterized by a core drug reservoir surrounded by a polymeric membrane.

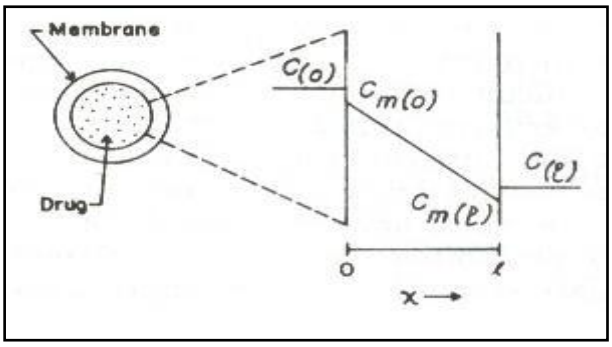

Figure 1.3: Systematic representation of diffusion controlled drug release matrix system.

Figure 1.3 Schematic representation of reservoir diffusion device Cm (o), and Cm (d) represent concentration of drug inside surfaces of membrane and C (o) & C (d) represents concentration in adjacent regions.

If it is assumed that the drug on the both side of membrane is in equilibrium with its respective membrane surface which in equilibrium between the membrane surfaces and their bathing solutions as shown in Figure 1.2

Therefore the concentration just inside the membrane surface can be related to the concentration in the adjacent region by following expression.

$K = Cm (o) / C(d)$ at $X = o$ (2)

$K = Cm (d) / C(d)$ at $X = d$ (3)

Where K = partition coefficient. If we consider K & D are constants then equation (1) becomes,

$$J = D K \Delta C/d \quad (4)$$

Where Δc is the concentration difference across the membrane and d is path length of diffusion. The simplest system to consider is that of slab, where drug release is from only one surface as shown Figure 1.2 in this case equation (4) becomes

$$dMt/ dt = ADK \Delta C/ d \quad (5)$$

The process of diffusion is generally described by Ficks equation,

$$J = -D\, dc/dx$$

Where, J -- Flux (amount/ area –time)

D -- Diffusion co-efficient of drug in the membrane (area / time)

Dc/dx -- rate of exchange in concentration C, with respect to a distance X in the membrane.

Advantages

1. Zero order delivery is possible.
2. Release rate variable with polymer type.

Disadvantages

1. Potential toxicity if system fails.
2. System must be physically removed from implant sites.
3. Difficult to deliver high molecular weight compounds.
4. Generally increased cost per dosage units.

b. Matrix Devices

It contains of drug dispersed homogeneously throughout a polymer matrix. In this model, drug in the outside layer exposed to bath solution is dissolved first and then diffuses out of the matrix. The following equation describe the rate of release of drug dispersed in an inert matrix system have been derived by Higuchi.

$$Dm/d_h = Cod_h – Cs/2.$$

Where, dm = Change in the amount of drug released per unit area.

d_h = Change in the thickness of the zone of matrix that have been depleted of drug.

Co = Total amount of drug in unit volume of matrix.

Cs = Saturated concentration of drug within the matrix.

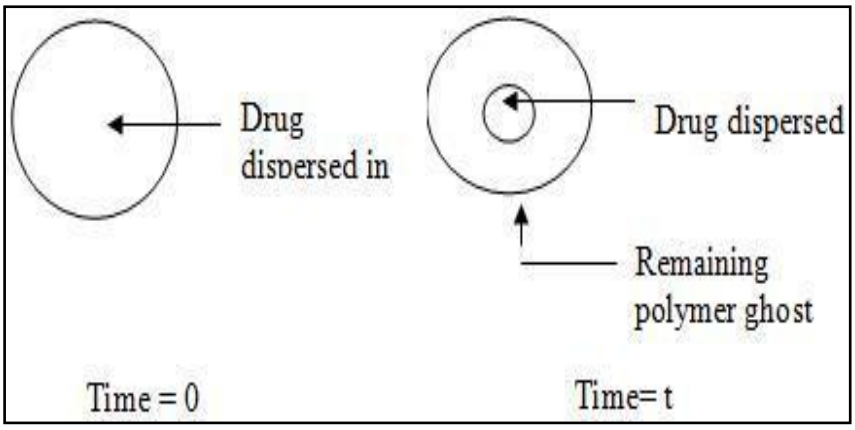

Figure1. 4: Release of drug dispersed in an inert matrix system.

Advantages

1. Can deliver high molecular weight compounds.
2. Easier to produce than reservoir devices.

Disadvantages

1. Removal of remaining matrix is necessary for implanted systems.
2. Cannot obtain zero order release.

Diffusion Rate Modifications

Modification of the following will change the rate of diffusion

(a) Thickness of the separating layer

(b) Porosity

(c) Partition coefficient

(d) Modification of the diffusion co-efficient.

(e) Modification of efficient molecular size.

(f) Modification of viscosity.

(g) Modification of concentration.

2. Dissolution-controlled Systems

Drug with a slow dissolution rate will demonstrate sustaining properties, since the release of the drug will be limited by rate of dissolution. This being the case, SR preparations of drugs could be made by decreasing their dissolution rate. This includes preparing appropriate salts or derivatives, coating the drug with a slowly dissolving material, or incorporating it into a tablet with a slowly dissolving carrier.

The dissolution process at steady state, is described by Noyes-Whitney equation,

$dc/dt = K_D A(Cs-C) = D/h\ A(Cs-C)$

Where,

dc/dt = Dissolution rate.

K_D = Diffusion co-efficient

A = surface area of the dissolving solid

Cs = Saturation solubility of the solid.

C = Concentration of solute in bulk solution.

H = Thickness of diffusion layer.

Principles of dissolution rate modification

The following are may affect dissolution rate modification of

(a) Solubility,

(b) Specific area,

(c) Particle shape and surface structure,

(d) Dissolution conditions (contact of solid particles with the Solvent) and

(e) Crystallographic modification.

3. Dissolution and Diffusion - Controlled release system

Normally, therapeutic systems will never be dependent on dissolution only or diffusion only. In practice, the dominant mechanism for release will over shadow other processes enough to allow classification as either dissolution rate limited or diffusion controlled.

The mechanism of release from simple erodible slabs, cylinders and spheres has been described by Hopenberg are described as

$$Mt/M = 1 - (1- K_o t/C_o a)^n$$

Where, n = 2 for cylinder and

n = 1 for a slab.

a = Radius of sphere or cylinder or half height of a slab.

M_t = Mass of drug release at time t

M = Mass released infinite time.

Advantages

1. Easier to produce than reservoir devices.
2. Can deliver high molecular weight compounds.
3. Removal from implant sites is not necessary.

Disadvantages

1. Difficult to control kinetics owning to multiple process of release.
2. Potential toxicity of degraded polymer must be considered.

4. Osmotically controlled systems

This device is fabricated as tablet that contains water soluble osmotically active drug, of that was blended with osmotically active diluents by coating the tablet with a cellulose triacetate barrier which functions as a semi permeable membrane. A laser is used to form a precision orifice in the barrier, through which the drug is released due

to development of osmotic pressure difference across the membrane, when this was kept in water.

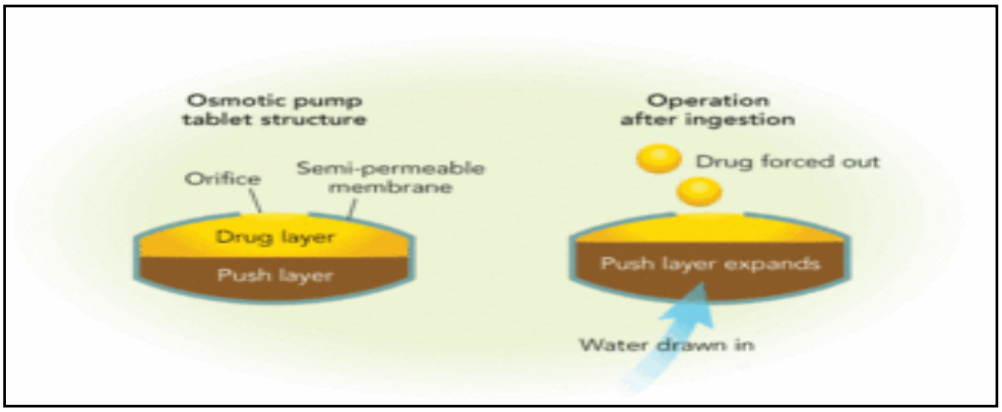

Figure 1.5: Osmotically controlled release systems.

5. Ion Exchange Systems

These are salts of cationic or anionic exchange resins or insoluble complexes in which drug release results from exchange of bound drug ions that are normally present in GI fluids.

1.4.MICROSPHERES:

(shobharani., 2008; Kedar Prasad Meena and Danji J.S., et al., 2011)

Microspheres are solid, approximately spherical particles ranging 1-1000µm in size. They are made up of polymeric substances, in which the drug is dispersed throughout the microsphere matrix. The substances used in the formulation are biodegradable synthetic polymers and natural products. The natural polymers of choice are albumin and gelatin, the synthetic ones being polylactic acid and polyglycolic acid. The polymers used to manufacture microspheres are chosen according to their solubility, stability profile, process, safety and economic suitability.

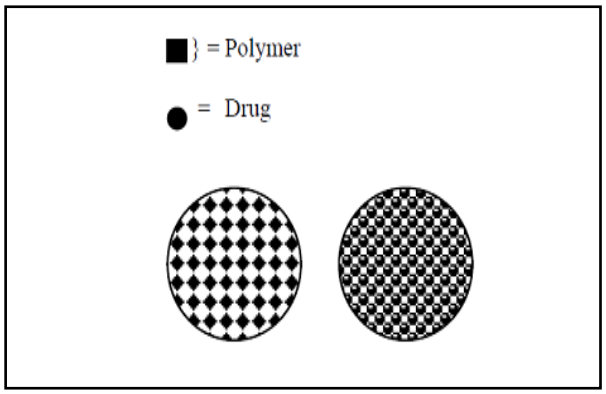

Fig 1.6: Microspheres

Advantages of microsphere delivery system:

- Protection of unstable, sensitive materials from their environments prior to use.
- Better process ability (improving solubility, dispersibility, flowability)
- Self-life enhancement by preventing degradative reactions.
- Safe and convenient handling of toxic materials.
- Masking of odor or taste.
- Enzyme and microorganism immobilization.
- Controlled and targeted drug delivery.
- Handling liquids as solids.
- To improve bioavailability
- To improve the stability
- Limiting fluctuation within therapeutic range

Applications of microspheres

Some of the applications of microspheres can be described in detail as given below

1. Prolonged release dosage forms. The microsphere drug can be administered, as microsphere is perhaps most useful for the preparation of tablets, capsules or parenteral dosage forms.

3. Microsphere can be used to prepare enteric coated dosage forms, so that the medicament will be selectively absorbed in the intestine rather than the stomach.
4. It can be used to mask the taste of bitter drugs.
5. From the mechanical point of view, microsphere has been used to aid in the addition of oily medicines to tablet dosage forms. This has been used to overcome problems inherent in producing tablets from otherwise tacky granulations. This was accomplished through improved flow properties. For example, the non-flowable multicomponent solid mixture of niacin, riboflavin, and thiamine hydrochloride and iron phosphate may be encapsulated and made directly into tablets.
6. It has been used to protect drugs from environmental hazards such as humidity, light, oxygen or heat. Microsphere does not yet provide a perfect barrier for materials, which degrade in the presence of oxygen, moisture or heat, however a great degree of protection against these elements can be provided. For example, vitamin A and K have been shown to be protected from moisture and oxygen through microsphere**.**
7. Microsphere can be used to decrease the volatility. An encapsulated volatile substance can be stored for longer times without substantial evaporation.
8. Microsphere has also been used to decrease potential danger of handling of toxic or noxious substances. The toxicity occurred due to handling of fumigants, herbicides, insecticides and pesticides have been advantageously decreased after microencapsulation.
9. The hygroscopic properties of many core materials may be reduced by microsphere.

10. Many drugs have been microsphere to reduce gastric irritation.
11. Microsphere method has also been proposed to prepare intrauterine contraceptive device.

1.5. Classification of polymers used for preparation of microspheres

(Vyas S.P., 2002)

A number of different substances both biodegradable as well as non-biodegradable have been investigated for the preparation of microspheres. These materials include the polymers of natural and synthetic origin and also modified natural substances. Some of the polymers used in the preparation of the microspheres are classified and listed below.

Types of polymers used in preparation of microspheres

Classification:

1] Synthetic polymer

A] Non biodegradable:

- Acrolein
- Glycid yl methacrylate
- Epoxy polymer
- PMMA

B] Biodegradable:

- Poly anhydrides
- Lactides and glycolides and their copolymer.
- Poly alkyl cyno acrylates

2] Natural Materials

A] Proteins

- Albumins
- Gelatins
- Collagens

B] Carbohydrates

- Chitosan
- Carrageenan
- Starch

C] Chemically modified carbohydrates

- Poly acryl starch
- Poly acryl dextran

1.6. TYPES OF MICROSPHERES

Bio adhesive microspheres:

Adhesion can be defined as sticking of drug to the membrane by using the sticking property of the water soluble polymers. Adhesion of drug delivery device to the mucosal membrane such as buccal , ocular , rectal, nasal etc can be termed as bio adhesion . These kinds of microspheres exhibit a pro longed residence time at the site of application and causes intimate contact with the absorption site and produces better therapeutic action.

Magnetic microspheres

This kind of delivery system is very much important which localizes the drug to the disease site. In this larger amount of freely circulating drug can be Replaced by smaller amount of magnetically targeted drug. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials that are used for magnetic microspheres are chitosan, dextran etc. The different type are Therapeutic magnetic microspheres: Are used to deliver chemo therapeutic agent to liver tumor. Drugs like proteins and peptides can also be targeted thro ugh this system.6 Diagnostic microspheres: Can be used for imaging liver metastases and also can be used to distinguish bowel lo ops from other abdominal structures by forming nano size particles supra magnetic iron oxides.

Floating micro spheres:

In floating types the bulk density is less than the gastric flu id and so remains buoyant in stomach without affecting gastric emptying rate. The drug is released slowly at the desired rate, if the system is floating o n gastric content and increases gastric residence and increases fluctuation in plasma concentration. Moreover it also reduces chances of striking and dose dumping. One another way it produces prolonged therapeutic effect and therefore reduces dosing frequencies. Drug (ketoprofen) given through this form.

Polymeric microspheres:

The different types of polymeric microspheres can be classified as follows and they are Bio degradable polymeric microspheres and Synthetic polymeric microspheres.

Biodegradable polymeric microspheres:

Natural polymers such as starch are used with the concept that they are bio degradable, Biocompatible, and also bio adhesive in nature. Biodegradable polymers prolongs the residence time when contact with mucous membrane due to its high degree o f swelling property with aqueous medium , results gel formation. The rate and extent of drug release is controlled by concentration of polymer and the release pattern in a sustained manner. The main drawback is, in clinical use drug loading efficiency of biodegradable microspheres is complex and is difficult to control the drug release. However they provide wide range of application in microsphere based treatment.

Synthetic polymeric microspheres:

The interest of synthetic polymeric microspheres are widely used in clinical application, More over that also used as bulking agent, fillers, embolic particles, drug delivery vehicles etc and proved to be safe and bio compatible. But the main disadvantage of these kinds of microspheres, are tend to migrate away from injection site and lead to potential risk, embolism and further organ damage.

Radioactive microspheres:

Radio emobolisation therapy microspheres sized 10-30nm is of larger than capillaries and gets tapped in first capillary bed when they come across. They are injected to the arteries that lead to tumour of interest. So all these conditions radioactive microspheres deliver high radiation dose to the targeted areas without damaging the normal surrounding tissues.

Prerequisites for ideal microsphere carriers:

The polymer utilized for the preparation of microspheres should ideally fulfill the following prerequisites

- ✓ Longer duration of action
- ✓ Control of content release
- ✓ Increase of therapeutic efficacy
- ✓ Protection of drug
- ✓ Reduction of toxicity
- ✓ Biocompatibility
- ✓ Sterilizability
- ✓ Relative stability
- ✓ Water solubility or dispersability
- ✓ Bioresorbability
- ✓ Targetability
- ✓ Polyvalent

1.7. PREPARATION METHOD OF MICROSPHERE

(Kedar Prasad Meena and Danji J.S., et al., 2011)

Preparation of microspheres should satisfy certain criteria

- ➢ The ability to incorporate reasonably high concentrations of the drug.
- ➢ Stability of the preparation after synthesis with a clinically acceptable shelf life.
- ➢ Controlled particle size and dispersability in aqueous vehicles for injection.
- ➢ Release of active reagent with a good control over a wide time scale.
- ➢ Biocompatibility with a controllable biodegradability.

METHODS USED FOR PREPARATION OF MICROSPHERES:

1. Protein gelation technique
2. Emulsion polymerization technique
3. Solvent evaporation technique
4. Soncation technique
5. Spray drying technique
6. Emulsion-heat stabilization technique
7. Spherical crystallization technique
8. Spray congealing
9. Phase separation coaservation method
10. Polymerization technique

1. Protein gelation technique

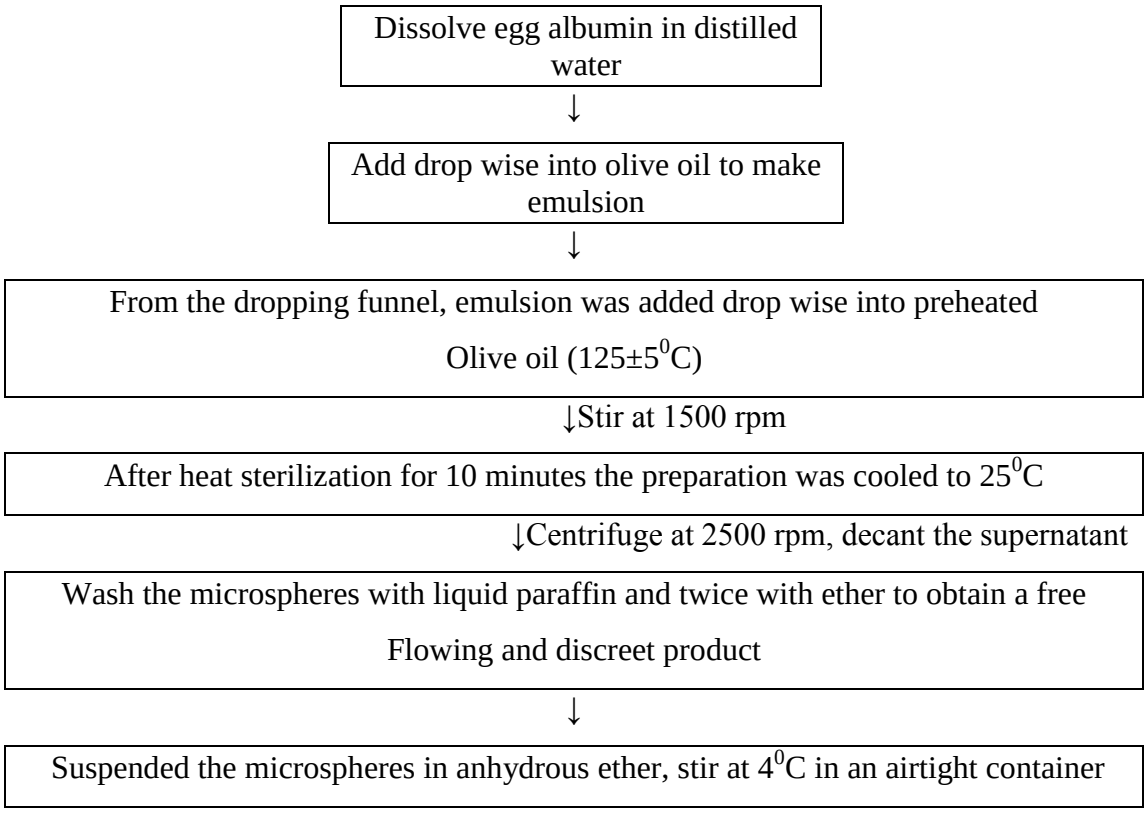

Figure 1.7: Preparation of microspheres by Protein gelation technique.

2. Emulsion polymerization technique

Take 100ml of light paraffin oil in glass beaker , mix with 0.4% w/v span 60

↓Stir and heat at 70^0C until complete solubilisation

Cool the mixture at room temperature

↓

Drop wise add 10ml of egg albumin aqueous solution of different drug to polymer ratio (1:1, 1:5, 1:2) using a 22-gauge hypodermic syringe into external phase

↓ Stir light paraffin at 600 rpm for 10 minutes

A w/o emulsion was formed

↓Raise the temperature of oil bath to 95^0C, stir until microspheres completely dehydrate

Microspheres thus obtained were decanted, washed 6 times with 20ml petroleum ether for 2 minutes at 700 rpm

↓ finally wash 3 times with 60 oz of distilled water for 2 minutes at 700 rpm, dry at room temp for 24 hour

After drying, a fine yellow free flowing powders of microsphere were obtained that was stored in desiccators at room temperature.

Figure 1.8: Preparation of microspheres by emulsion polymerization technique.

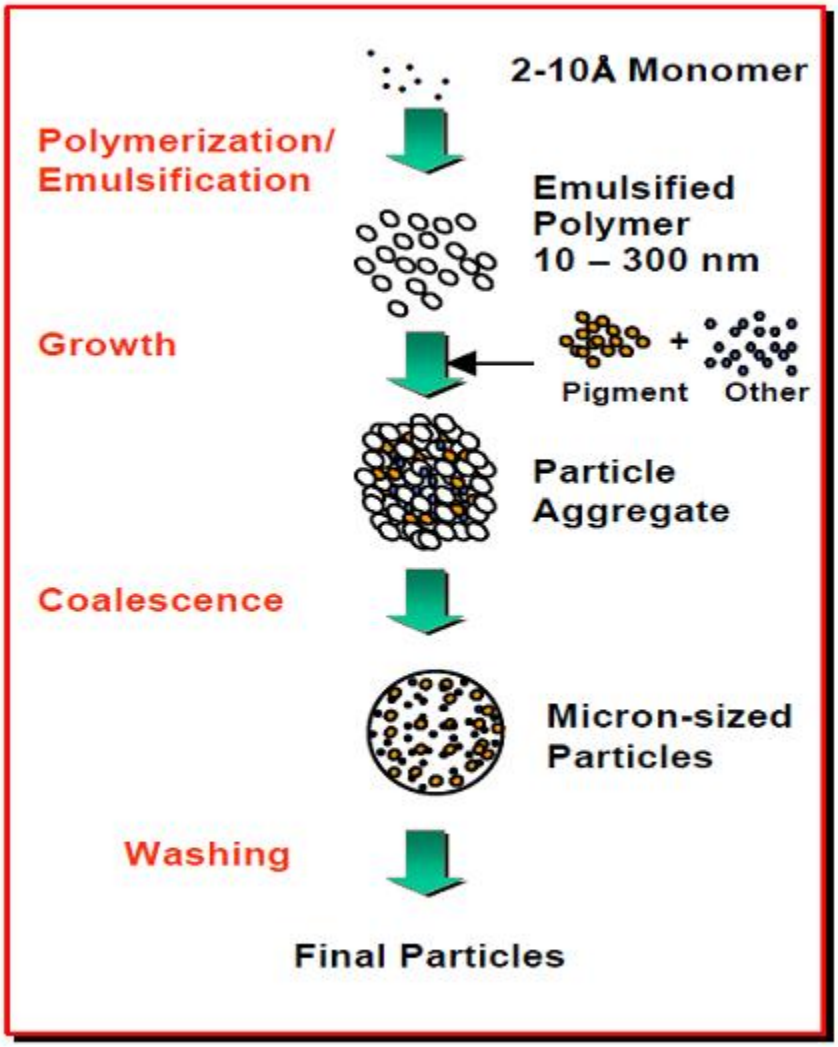

Figure 1.9: Microsphere preparation by emulsion method

3. Solvent evaporation technique

A core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation the core material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size microsphere. The mixture is then heated if necessary to evaporate the solvent. The solvent Evaporation technique to produce microspheres is applicable to wide variety of core materials. The core materials may be either water soluble or water insoluble materials. Solvent evaporation involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous.

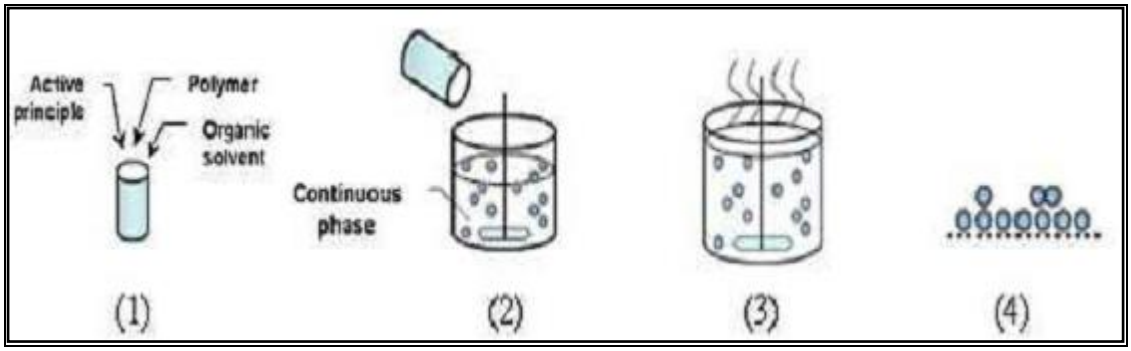

Figure 1.10: Basic steps of microsphere by solvent evaporation method

4. Sonication technique:

As the technique name itself is self explanatory, it just involves a simple sonication for certain period of time till a desired size of microspheres are obtained. The polymer solution of desired concentration is taken which is sonicated. To this add the drug which will then form intrachain cross-link with cysteine residues of polymer chains. Prepared a stable preparation of air filled human polymer microspheres (Albunex) by sonication technique. The microspheres ranged in size from 1-10m with 99% of particles smaller than 10 m. The mean size was 5 m, which is small enough to pass freely through the pulmonary capillary circulation.

5. Spray drying technique:

In Spray Drying the polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, Acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high-speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporate instantaneously leading the formation of the microspheres in a size range 1-100m.

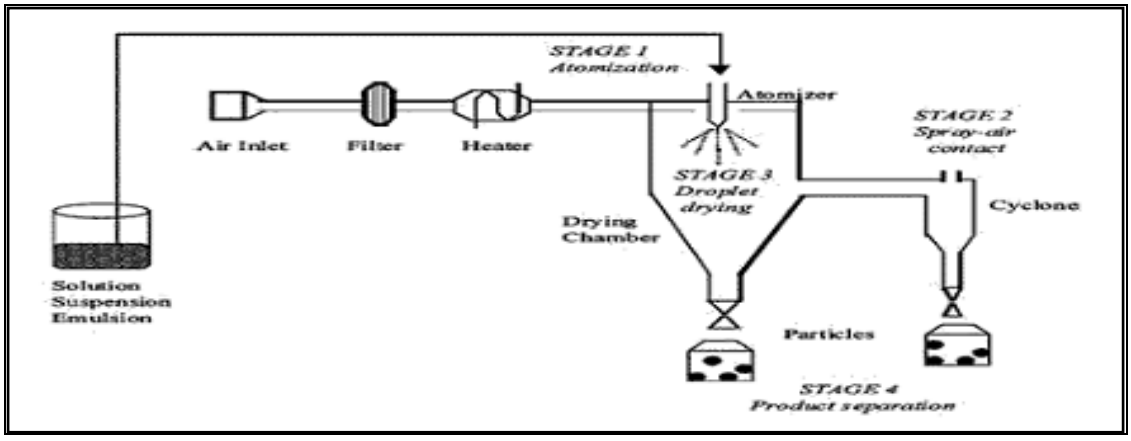

Figure 1.11: Main process stages involved in spray drying process.

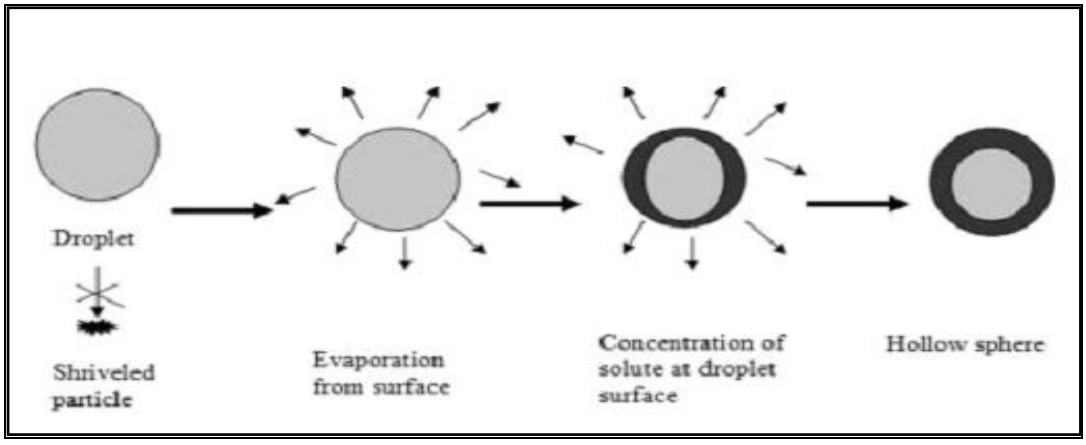

Figure 1.12: Formation of product in spray drying.

Principle

There are three fundamental steps involved in spray drying

1) Atomization of a liquid feed into fine droplets.

2) Mixing of these spray droplets with a heated gas stream, allowing the liquid to evaporate and leave dried solids.

3) Dried powder is separated from the gas stream and collected. Spray drying involves the atomization of a liquid feedstock into a spray of droplets and contacting the droplets with hot air in a drying chamber.

The sprays are produces by either rotary (wheel) or nozzle atomizers. Evaporation of moisture from the droplets and formation of dry particles proceed under controlled temperature and airflow conditions. Powder is discharged continuously from the drying chamber. Operating conditions and dryer design are selected according to the drying characteristics of the product and powder specification.

6. Emulsification-heat stabilization technique

5% solution of BSA containing 0.1% Tween80 was made, to which 4% drug was added and used as the aqueous phase. The oil phase composed of 30 ml maize oil and 10 ml petroleum ether with 1% Span 80 as emulsifier were mixed together and allowed to stir for 10 min at 1000 rpm. The aqueous phase was added drop wise to the oil phase and stirred on a magnet stirrer at 1000 rpm for 30 min to form the initial emulsion. This emulsion was then added to 40 ml of maize oil preheated to 120° C and stirred at 1000 rpm for 15 min to allow the formation and solidification of microspheres. The microsphere suspension was centrifuged at 3500 rpm for 30 min and the settled microspheres were washed three times with ether to remove traces of oil on microsphere surfaces. The microspheres were vacuum dried in desiccators overnight and stored were vacuum dried in a desiccators overnight and stored at 4°C in dark. The microspheres had mean diameters between 1-25 m of which more than 50 percent were below 5 m.

7. Spherical crystallization technique.

Development and characterization of sustained release microspheres by solvent diffusion method. The microspheres were prepared using the emulsion solvent diffusion method of the spherical crystallization technique. Drug and polymer were dissolved completely in the acetone–dichloromethane mixture. Then Aerosil was

suspended uniformly in the drug– polymer solution under vigorous agitation. The resultant drug–polymer– Aerosil suspension was poured into the distilled water (150 ml) containing 0.08% of SDS (i.e. poor solvent) under a moderate agitation (450–750rpm) and thermally controlled at 0–38°C. The suspension was finely dispersed into emulsion droplets immediately under agitation, and the drug and polymers co precipitated in the emulsion droplets. After agitating the system for 20 min, 150 ml of poor solvent was added slowly to promote the diffusion of the good solvent from emulsion droplets into poor solvent resulting in enhancement of the solidification of emulsion droplets.

8. Spray congealing

The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high speed homogenization. This dispersion is then atomized in a stream of cold air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of the microspheres in a size range 1-100 m.

9. Phase separation coacervation technique

This process is based on the principle of decreasing the solubility of the polymer in organic phase to affect the formation of polymer rich phase called the coacervates. In this method, the drug particles are dispersed in a solution of the polymer and an incompatible polymer is added to the system which makes first polymer to phase separate and engulf the drug particles. Addition of non-solvent results in the solidification of polymer. Poly lactic acid (PLA) microspheres have been prepared by this method by using butadiene as incompatible polymer. The process variables are very important since the rate of achieving the coacervates

determines the distribution of the polymer film, the particle size and agglomeration of the formed particles. The agglomeration must be avoided by stirring the suspension using a suitable speed stirrer since as the process of microspheres formation begins the formed polymerize globules start to stick and form the agglomerates. Therefore the process variables are critical as they control the kinetic of the formed particles since there is no defined state of equilibrium attainment

10. Polymerization techniques

The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as:

I. Normal polymerization

II. Interfacial polymerization. Both are carried out in liquid phase.

- **Normal polymerization**

It is carried out using different techniques as bulk, suspension, precipitation, emulsion and micellar polymerization processes. In bulk, a monomer or a mixture of monomers along with the initiator or catalyst is usually heated to initiate polymerization. Polymer so obtained may be moulded as microspheres. Drug loading may be done during the process of polymerization. Suspension polymerization also referred as bead or pearl polymerization. Here it is carried out by heating the monomer or mixture of monomers as droplets dispersion in a continuous aqueous phase. The droplets may also contain an initiator and other additives. Emulsion polymerization differs from suspension polymerization as due to the presence initiator in the aqueous phase, which later on diffuses to the surface of micelles. Bulk polymerization has an advantage of formation of pure polymers.

- **Interfacial polymerization**

It involves the reaction of various monomers at the interface between the two immiscible liquid phases to form a film of polymer that essentially envelops the dispersed phase

Loading of drug: *(vyas s.p., 2002)*

The active components are loaded over the microspheres principally using two methods. i.e. during the preparation of the microspheres or after the formulation of the microsphere by incubating them with the drug/protein. The active component can be loaded by means of the physical entrapment, chemical linkage and surface absorption. The entrapment largely depends on the method of preparation and nature of the drug or polymer.

Maximum loading can be achieved by incorporating the drug during the time of preparation but it may get affected by many other process variables such as method of preparation, presence of additives, heat of polymerization, agitation intensity, etc.

The loading is carried out in pre-formed microspheres by incubating them with high concentration of the drug in a suitable solvent. The drug in these microspheres loaded via penetration or diffusion of the drug through the pores in the microspheres as well as adsorption on the surface. The drugs and protein can also be incorporated by physical or chemical linkage. The absorption of the drug/proteins depends on the nature of the polymers.

1.8. DRUG RELEASE KINETICS

Release of the active constituent is an important consideration in case of microspheres. Many theoretically possible mechanisms may be considered for the release of drug from the microspheres.

1. Liberation due to polymer erosion or degradation,
2. Self diffusion through the pore,
3. Release from the surface of the polymer,
4. Pulsed delivery initiated by the application of an oscillating or sonic field.

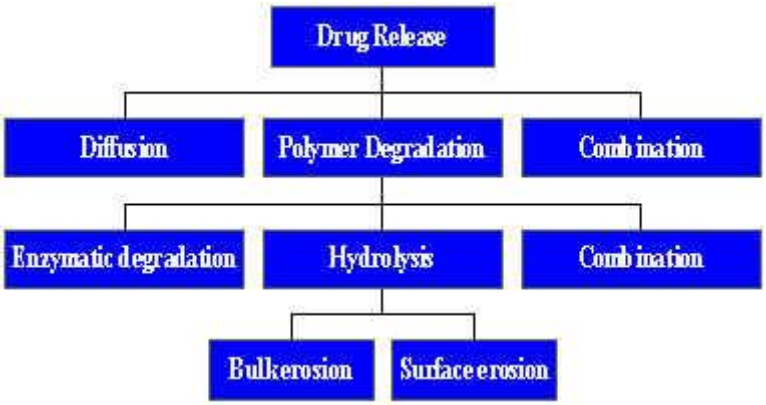

Figure 1.13: Possible drug release mechanisms for polymeric drug delivery

FACTORS INFLUENCING PROPERTIES OF MICROSPHERES. :

(Kedar Prasad Meena and Danji J.S.,et al., 2011)

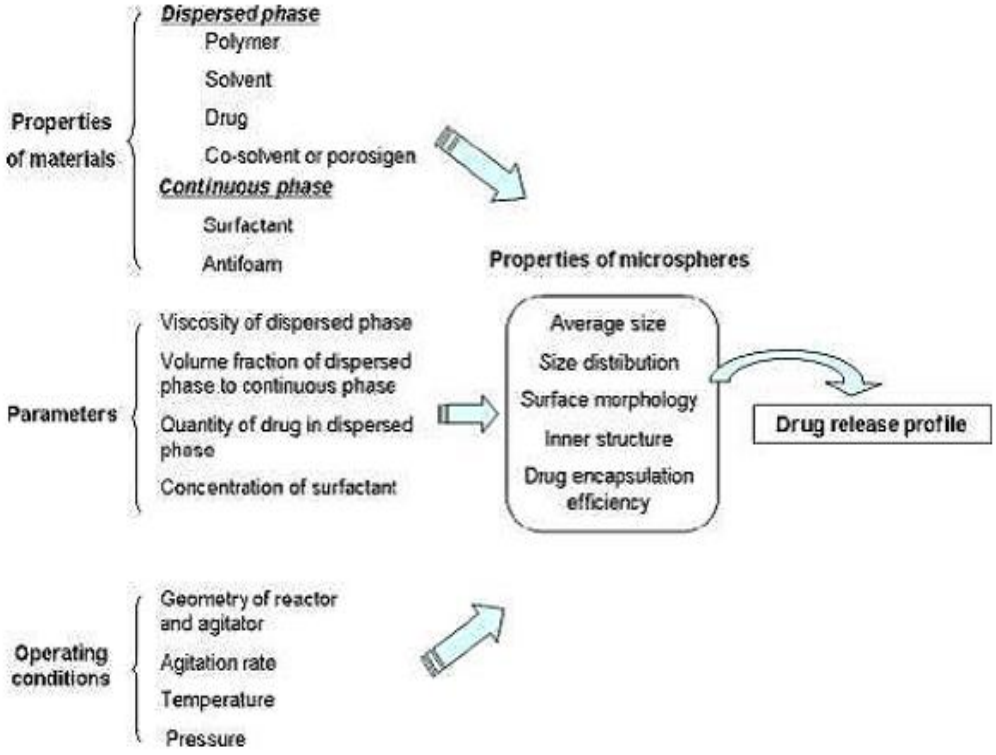

Figure 1.14: Factors effecting properties of microspheres

(A) Dispersed phase

1. Polymers commonly used to form microspheres

2. Choice of solvent

(1) Should be able to dissolve the chosen polymer;

(2) Poorly soluble in the continuous phase;

(3) High volatility and a low boiling point;

(4) Low toxicity.

(5) Alternative components (dispersed phase)

(a) Co-solvent:- organic solvents miscible with water such as methanol and ethanol.

(b) Porosity generator:- increases the degradation rate of polymer and improves drug release rate. E.g. Incorporating sephadex (cross-linked dextran gel) into insulin–pla microspheres significantly increases microsphere porosity.

(B) Continuous phase

(a) Surfactant:-

- It reduces the surface tension of continuous phase.
- Avoids the coalescence and agglomeration of drops.
- Stabilizes the emulsion.
- Widely used stabilizers include:

i. Non-ionic: partially hydrolyzed poly vinyl alcohol, methylcellulose, tween, span

ii. anionic: sodium dodecyl sulphate (sds), sodium lauryl sulphate.

iii. cationic: cetyltrimethyl ammonium bromide (ctab).

(b) Alternative component:-

- Antifoaming agent - foaming problem will disturb the formation of microspheres.
- Anti-foams of silicon and non-silicon constituents are used.

(C) Impact of parameters and operating conditions on the properties of microspheres.

Technology limitations in preparing microspheres

- Residual solvents
- Stability
- Non availability of degradable, synthetic polymers
- Encapsulation efficiency
- Limitation of manufacturing process
- Sterilization

1.9. HYPERTENSION (*http://www.hypertension.com*)

High blood pressure, termed "hypertension," is a condition that afflicts almost 1 billion people worldwide and is a leading cause of morbidity and mortality. More than 20% of Americans are hypertensive, and one-third of these Americans are not even aware they are hypertensive. Therefore, this disease is sometimes called the "silent killer." This disease is usually asymptomatic until the damaging effects of hypertension (such as stroke, myocardial infarction, renal dysfunction, visual problems, etc.) are observed. Hypertension is a major risk factor for coronary artery disease, myocardial infarction ("heart attacks") and stroke.

Definition of Hypertension:

Hypertensive is defined as an abnormal elevation in diastolic pressure and/or systolic pressure; mean arterial pressure is also elevated in hypertension, but it is not usually measured in people. In past years, the diastolic value was emphasized in assessing hypertension. However, elevations in systolic pressure ("systolic hypertension") are also associated with increased incidence of coronary and cerebrovascular disease (e.g., stroke).

Table: 1.1 Classification of hypertension

Classification	Systolic (mmHg)	Diastolic (mmHg)
Normal	<120	<80
Pre hypertension	120-139	80-89
Stage 1	140-159	90-99
Stage 2	>160	>100

Types of High Blood Pressure:

High blood pressure (also called hypertension) based on cause and characteristics.

- Essential Hypertension
- Isolated Systolic Hypertension
- Secondary Hypertension
- White-Coat Hypertension
- Labile Hypertension
- Malignant Hypertension
- Hypertension During Pregnancy

Essential Hypertension

About 90 to 95 percent of people with high blood pressure have essential hypertension or primary hypertension. This means the condition has no identifiable medical cause as its source. Essential hypertension is often inherited. Elevated blood pressure usually begins to appear between age 30 and 50, but can begin at older ages.

Without a definite understanding of what causes essential hypertension, doctors often explain it as a malfunction of one or more parts of the blood-pressure regulatory system. Different factors increase blood pressure in different people. That's why a treatment that lowers blood pressure in one person does not always work in another. For example, a person who is "salt sensitive" usually can control his or her blood pressure with a low-salt (sodium) diet alone, while another person may find salt intake has little or no influence on blood pressure.

Isolated Systolic Hypertension

As people age, their arteries tend to lose elasticity and become less able to accommodate blood surges, leading to hardening of the arteries (arteriosclerosis). Hardening of the arteries can elevate systolic blood pressure (the top number of your blood pressure), while diastolic pressure (the bottom number) stays in the normal range. This condition, called isolated systolic hypertension, is the most common form of high blood pressure in the elderly.

The Framingham Heart Study, which has tracked the health of participants since the late 1940s, found that 65 percent to 75 percent of people over age 65 with elevated blood pressure had isolated systolic hypertension.

In the past, doctors considered this type of high blood pressure to be normal in elderly patients, so normal that it wasn't even treated. However, a 1991 study, Systolic Hypertension in the Elderly Program (SHEP), provided strong evidence to the contrary. SHEP tracked 4,736 patients with isolated systolic hypertension over five years. Half the participants used drugs to lower their blood pressure, while the other half received a placebo (an inactive pill). The group taking medication had significantly fewer strokes and heart attacks than the placebo group. The SHEP study has spurred doctors to treat isolated systolic hypertension in older patients more aggressively.

Secondary Hypertension

Some medical conditions and medications can cause high blood pressures. This is called secondary hypertension, because high blood pressure is the second thing to develop, after the initial medical condition. Often, if the medical problem can be identified and treated, the high blood pressure will come back down to a normal level.

Your doctor may suspect secondary hypertension if your blood pressure if very difficult to control with medicines.

White-Coat Hypertension

Anxiety can raise blood pressure. That's why some people who have a normal blood pressure at home find that their blood pressure is high when they see a doctor. This phenomenon is called "white-coat hypertension."

If you or your doctor thinks you have "white-coat hypertension," you can use a home blood-pressure machine to check your blood pressure periodically over the course of a week or two.

Labile Hypertension:

Labile hypertension is blood pressure that fluctuates abruptly and repeatedly, often causing symptoms such as headache or ringing in the ears. People sudden increase in your blood pressure. With labile hypertension often react to emotional stress with an increase in blood pressure. Frequently, traditional blood-pressure medicines have little effect. Some people with labile hypertension require anti-anxiety medications in order to gain control of their blood pressure.

People who are habitually affected by stress — whether from losing a job, feeling pressure at work or simply getting stuck in traffic — may develop high blood pressure that could inflict some of the same damage as full-time hypertension.

Malignant Hypertension

Though rare, malignant hypertension is the most threatening form of high blood pressure. It's marked by an unusually sudden rise in blood pressure to dangerous levels. Diastolic pressure often reaches 130 or higher. However, malignant

hypertension may also occur at lower, less alarming levels, if the rise is particularly sudden. Unlike other kinds of high blood pressure, malignant hypertension is usually accompanied by dramatic symptoms such as severe headache, shortness of breath, chest pain, nausea and vomiting, blurred vision, or even blindness, seizures and loss of consciousness.

Malignant hypertension is an emergency. Anyone with malignant hypertension must be hospitalized immediately. It places people at immediate risk for heart attack, stroke, heart failure, permanent kidney damage, bleeding into the brain (hemorrhagic stroke) and brain swelling.

Malignant hypertension develops in less than 1 percent of people who already have high blood pressure. Rarely, the appearance of malignant hypertension is the first sign that a person has high blood pressure. The cause of this condition is usually unknown, but occasionally it can be a reaction of your body to a drug of abuse, like cocaine, or a reaction to stopping a blood-pressure medicine.

Hypertension during Pregnancy:

High blood pressure occurs in 6 to 8 percent of pregnancies, and in most of these cases, it is diagnosed during a first pregnancy. Pregnancy can cause high blood pressure due to hormonal changes or from a serious complication of pregnancy known as preeclampsia, a condition that causes tightening of arteries throughout the mother's body and placenta, as well as unpredictable blood clotting.

High blood pressure in the first half of a pregnancy (the first 20 weeks) is called chronic hypertension in pregnancy. High blood pressure in the second half of pregnancy (weeks 20 through 40) could be any of the following:

1. **Chronic hypertension:** in pregnancy is high blood pressure caused by a condition unrelated to pregnancy (such as essential hypertension or secondary hypertension) that begins or continues during pregnancy. The rise in blood pressure may have predated the pregnancy by months or years. However, this rise is first noticed during pregnancy, when a woman who has not regularly visited a doctor gets prenatal care. Chronic hypertension in pregnancy continues after the baby is delivered. Women with chronic hypertension in pregnancy are at increased risk for developing preeclampsia.
2. **Gestational hypertension** (or pregnancy-induced hypertension) is high blood pressure that results from the effects of the hormone estrogen. Blood pressure returns to normal within 12 weeks after the baby is delivered.
3. **Preeclampsia** (or toxemia of pregnancy) causes tightening of arteries throughout the mother's body and placenta, as well as unpredictable blood clotting. It not only creates high blood pressure but also causes fluid retention that leads to swelling of the feet and legs (and sometimes the hands and face), and protein in the urine. Preeclampsia can progress to cause neurological symptoms including seizures. Preeclampsia requires very close attention from your doctor and frequently requires the early delivery of the baby in order to keep both mother and baby safe from harm.

Sometimes it's not possible to know what causes blood pressure to rise during pregnancy until the pregnancy is over. Protein in the urine any time during pregnancy or in the first 12 weeks after delivery confirms preeclampsia. If high blood pressure goes away within 12 weeks of delivery, and there was never significant protein in the urine, the cause can be diagnosed as gestational hypertension. If high blood pressure does not go away after delivery, chronic hypertension is the culprit. It is even possible to have two causes of high blood pressure during pregnancy. For example, a woman with chronic hypertension may develop preeclampsia in the second half of her pregnancy.

Etiology & Classification:

- ✓ Primary (Essential) Hypertension
  1. Sympathetic nervous system hyperactivity
  2.Abnormal cardiovascular development
  3.Renin- angiotensin system activity
  4.Defect in natriuresis
  5.Intracellular sodium and calcium
  6.Exacerbating factors
- ✓ Secondary Hypertension
  1.Renal disease
  2.Genetic causes
  3.Renal vascular hypertension
  4. Primary hyperaldosteronism
  5 Cushing's syndrome
  6. Pheochromocytoma
  7. Coarctation of the aorta

8. Hypertension associated with pregnancy
9. Estrogen use
10. Other causes of secondary hypertension

Signs and symptoms of hypertension:

Signs and symptoms of pulmonary hypertension (PH) may include:

- Shortness of breath during routine activity, such as climbing two flights of stairs
- Tiredness
- Chest pain
- A racing heartbeat
- Pain on the upper right side of the abdomen
- Decreased appetite

As PH worsens, you may find it hard to do any physical activities. At this point, other signs and symptoms may include:

- Feeling light-headed, especially during physical activity
- Fainting at times
- Swelling in your legs and ankles

Treatment of Hypertension:

Most people with hypertension are treated with antihypertensive medications. In most forms of hypertension, the hypertensive state is maintained by an elevation in blood volume, which in turn increases cardiac output by the Frank-Starling relationship. Diuretic drugs, which enhance the removal of sodium and water by the kidneys and thereby decrease blood volume, are very effective in the treatment of hypertension. Hypertension is also commonly treated with drugs that decrease cardiac output. These

cardioinhibitory drugs either block beta-adrenoceptors on the heart (i.e., beta-blockers) or L-type calcium channels (i.e., calcium-channel blockers), which decreases cardiac output by decreasing heart rate and contractility (inotropy). Vasodilator drugs, which decrease systemic vascular resistance, are also used to treat hypertension. Included in these drugs are alpha-adeno receptor antagonists (alpha-blockers), direct-acting vasodilators, angiotensin -converting enzyme inhibitors and angiotensin receptor blockers

Prevention of Hypertension:

Much of the disease burden of high blood pressure is experienced by people who are not labelled as hypertensive. Consequently, population strategies are required to reduce the consequences of high blood pressure and reduce the need for antihypertensive drug therapy. Lifestyle changes are recommended to lower blood pressure, before starting drug therapy. The following are the primary preventive measures of hypertension:

- maintaining normal body weight for adults (e.g. body mass index 20–25 kg/m^2)
- reducing dietary sodium intake to <100 mmol/ day (<6 g of sodium chloride or <2.4 g of sodium per day)
- engage in regular aerobic physical activity such as brisk walking (≥30 min per day, most days of the week)
- limit alcohol consumption to no more than 3 units/day in men and no more than 2 units/day in women
- consume a diet rich in fruit and vegetables (e.g. at least five portions per day)

LITERATURE SURVEY...

2. LITERATURE SURVEY

2.1. Literature Review:

Recent advancement in Microsphere drug delivery systems:

1) Anuj chawala., ***et al.*** **(2012**) was to prepare site specific drug delivery of naproxe sodium using sodium alginate and Eudragit S-100 as a mucoadhesive and pH-sensitive polymer, respectively Core microspheres of alginate were prepared by a modified emulsification method followed by cross-linking with CaCl2, which was further coated with the pH dependent polymer Eudragit S-100 (2.5 or 5 %) to prevent drug release in the upper gastro intestinal environment. Drug release from all sodium alginate microsphere formulations followed Higuchi kinetics. Moreover, drug release from Eudragit S-100 coated microspheres followed the Korsmeyer-Peppas equation with a Fickian kinetics mechanism.

2) Bipul nath. , ***et al.*** **(2012)** objective of present investigation was to evaluate the entrapment efficiency of the anti-HIV drug, zidovudine, using two Eudragit polymers of different permeability characteristics and to study the effect of this entrapment on the drug release properties. The release rate of zidovudine from Eudragit RS 100 microspheres was much lower than that from Eudragit RL 100 microspheres. Evaluation of release data reveals that release of zidovudine from Eudragit RL 100 microspheres followed the Higuchi rule, whereas Eudragit RS 100 microspheres exhibited an initial burst release, a lag period for entry of surrounding dissolution medium into polymer matrix and finally, diffusion of drug through the wall material.

3) Bhaskar mazumder., *et al*. (2012) aim of this study is to prepare and characterize the microspheres of chlorpheniramine maleate (CPM) with the combination of cellulose derivatives: ethyl cellulose and cellulose acetate. Microspheres were prepared with the combination of ethyl cellulose and cellulose acetate using oil-in-oil emulsion solvent evaporation method. Microspheres were characterized by particle size analysis, percentage yield, and entrapment efficiency, scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy, *In-vitro* release studies and release kinetics. The analysis of dissolution kinetic data shows that it follows Higuchi model then zero order followed by first order.

4) Khaled M., *et al*. (2012) aim of this work was to formulate and evaluate controlled-release oxypentifylline (OXP) microspheres that fulfill the requirements for extended release medications using Ammonio Methacrylate Copolymer RS100 as polymeric material. The microspheres were prepared by quasi-emulsion solvent diffusion technique. The effect of process variables such as a drug to polymer ratio, stirring rate, and concentration of emulsifier on mean particle size, yield, entrapment efficiency and *in-vitro* release characteristics of microspheres were studied**.** This study showed that Ammonio Methacrylate Copolymer RS100 can be used successfully to sustain the release of OXP.

5) Prasanth Singh., *et al*. (2012) the present work is to formulate and evaluate Ivabradine HCl (IBH) microspheres using egg albumin with the aim to get the best possible drug- polymer ratio giving the sustained drug release. IBH loaded egg albumin microspheres were prepared by heat denaturation technique. Various evaluation parameters were assessed, with a view to obtain sustained release of drug. The prepared IBH microspheres were then subjected to FTIR,

SEM, particle size and size distribution, % yield, % drug loading, entrapment efficiency, *In-vitro* dissolution studies, release kinetics and DSC. The *in-vitro* dissolution data maximum of 73.26% cum. drug release was obtained in the IBH loaded microspheres. The *in-vitro* performance of IBH microspheres showed that sustained release was dependent upon the polymer concentration.

6) T.S Keerthi., *et al*. (2012) studied about design and characterization of microspheres of anti-hypertensive drug (losartan) using biodegradable natural polymer. The particle size distribution, entrapment efficiency and their release profile were investigated. Hence the drug release from the microspheres in a controlled manner in a regular fashion over extended period of time in comparision to all formulations. The microspheres found to be sustained for 12 hours and successful for oral drug delivery.

7) T. Sudhamani., *et al*. (2012) was formulated Ibuprofen microspheres by using Ethyl cellulose as carrier. These ethyl cellulose microspheres were prepared by the solvent evaporation method. The prepared microspheres were subjected to various evaluations and *in- vitro* release studies. Highest percentage of loading was obtained by increasing the amount of ibuprofen with respect to polymer. The particle sizes of the prepared microspheres were determined by optical microscopy and SEM analysis. The prepared microspheres had good spherical geometry with smooth surface as evidence by SEM.

8) Vidhya Kumari., *et al*. (2012) The main objective of this study was to formulate, develop and characterize Ofloxacin microspheres to prolong the release rate so as to decrease the necessity of multiple dosings especially in patients with renal impairment. The Ofloxacin microspheres were prepared using natural polymers by non-ionic cross linking technique. The prepared microspheres were

evaluated for percentage drug loading, entrapment efficiency, surface morphology, and *in-vitro* release characteristics to identify the effect of addition of these polymers .Cumulative release data were fitted into kinetic models Drug loading was found to increase with the increase in the concentration of encapsulating polymer, chitosan, sodium alginate and gelatin concentration. Drug release obeyed the first order kinetics.

9) G. Dinesh babu., ***et al.*** **(2011)** was prepared and evaluated the Diclofenac potassium (DP) microspheres were prepared by using Double Emulsion-solvent evaporation method with ethyl cellulose (EC) and Eudragit polymers. By using different formulation variables, eight different formulations were prepared. The resulting microspheres obtained, were more spherical in shape and showed more entrapment efficiency. The size of the microspheres varied between 346-695 μm and as high as 96.48% loading efficiency for Eudragit and 77.36% for EC was obtained. In-vitro release study was carried out EC microspheres released 23% of drug and Eudragit released 7% of drug. After 4 hours of dissolution 78% diclofenac potassium was released from EC microspheres and 92.35% of drug was released from Eudragit microspheres.

10) Hongfei Liu., ***et al.*** **(2011)** was to fabricate novel ambroxol hydrochloride carboxy methyl chitosan microspheres were investigated by X-ray diffraction; the results showed that the drug chemically bonds to the ion exchangeable structure of the microspheres. The evaluation of the microspheres was investigated by dynamic light scattering, scanning electron microscopy (SEM) and UV spectrophotometer. Finally the In-vitro drug release was tested in different ionic concentration dissolution medium the results showed that the microspheres had a controlled release profile for 8 h In-vitro without obvious

burst release.

11) Mohan raj palanisamy., *et al*. (2011) was developed to optimize the drug release pattern of a sustained release dosage form of metoprolol succinate. The preparation of MS (metoprolol succinate) microspheres was carried out using poly (ε-caprolactone) at different polymer: drug ratios from 1: 1 to 7: 1 and 10: 1 (F1 to F7 and F8) by the emulsion-solvent evaporation method. Physico chemical characterization of different ratios of formulations (F1 to F8) of microspheres was carried out such as the drug content, drug entrapped, particle size, bulk density and angle of repose. The *In-vitro* drug release over 24 h was performed by the method specified in USP XXIV. It was found that the drug release kinetics followed the Korsmeyer–Peppas equation. This approach optimizes the properties of the sustained release microspheres of metoprolol succinate, which can minimize both drugdose, and frequency of dosing.

12) Rahul nair., *et al*. (2011) aimed to prepare and characterize mefenemic acid microspheres using chitosan prepared by emulsion cross linking method to improve the oral bioavailability by reducing the gastrointestinal and renal effects. The in vivo studies of microspheres in albino rats demonstrated significant analgesic and anti-inflammatory activities of microspheres for longer period of time successfully.

13) Chinna Gangadhar B., *et al*. (2010) The aim of this study was to formulate and evaluate microspheres as controlled release preparations of a highly water-insoluble drug, Indomethacin, using natural polymer, Egg albumin; semi synthetic polymer, Ethyl cellulose and Synthetic polymer, Meth acrylic acid esters (Eudragit L 100) as the retardant materials. Microspheres were prepared by solvent evaporation method and Phase separation co-acervation method. The

prepared microspheres were evaluated for their micromeritic properties, drug content and encapsulation efficiency and characterized by Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM). The In-vitro release studies was performed by buffer change method pH 1.2, carbonate buffer (acidic) and pH 7.4, phosphate buffer (Alkaline). The best-fit release kinetics was achieved with Koresmeyer-Peppas plot followed by zero order and First order. The release of Indomethacin was influenced by the drug to polymer ratio and particle size &was found to be both diffusion and dissolution controlled.

14) Gambhire Makarand., *et al*. (2010) was studied the preparation and physico- chemical evaluation of Rifampicin microspheres . Rifampicin has poor and variable bioavailability. The present investigation was aimed to develop RIF loaded porous microspheres as a controlled release dosage form. Eudragit based porous microspheres of RIF were prepared by emulsion solvent diffusion method. Prepared porous microspheres were evaluated for its entrapment efficacy, morphology, thermal behavior, crystalline nature, in-vitro drug release and stability in simulated gastric fluid. The results obtained from the present investigation concluded that Rifampicin loaded porous microspheres are suitable for developing oral controlled release dosage form.

15) Karthikeyan., *et al*. (2010) was formulated microspheres with Metoprolol tartrate was to micro encapsulate the Anti-Hypertensive drug was to provide sustained release and maintain constant the plasma drug concentration .Metoprolol was microencapsulated with Eudragit-RS100 using solvent evaporation techniques. The effect of different formulation variables including the drug polymer ratio. Metoprolol microspheres was subjected to

micromeritic properties including the Angle of Repose, Bulk density, Carr's index, Haugner's ratio and Particle size determination. Metoprolol microspheres were subjected to Drug Loading, invitro drug release as well as for SEM and kinetic drug release.

16) A.R. Shabaraya., *et al.* (2009) was aimed to prepare Metoprolol tartrate was formulated as biodegradable microspheres using chitosan by the phase separation emulsification technique. Microspheres of drugs to carriers were prepared and thermally cross-linked. The size range of the microspheres varies from3.5 to 31.5µm. UV and DSC studies were carried out to confirm the presence and stability of the drug in the microspheres. Short-term stability studies were carried out at different temperatures. In-vitro release studies were carried out at different pH for a period of 10 h and compared with pure drug. The release of metoprolol tartrate from the chitosan microspheres was found to be sustained.

17) A.Balaiah., *et al.* (2008) was to prepare oral microspheres of levetiracetam with a view to reduce the frequency of dosing and to attain steady state drug levels. Levetiracetam is a second-generation antiepileptic agent useful in the treatment of partial onset and myoclonic seizures. Microspheres are suitable drug delivery system for such drug candidate. Microspheres were prepared by various methods like w/o/o double emulsion solvent diffusion and w/o/w double emulsion solvent evaporation technique. Pre formulation studies were carried out to rule out any drug-polymer interactions by DSC technique. In the In-vitro release studies initial burst release was observed from all the formulations. The data obtained from In-vitro release showed highest correlation with Higuchi model and the drug release was found to be diffusion controlled.

18) S K Basu., ***et al*. (2008**) Nitrendipne-loaded Eudragit RL 100 microspheres were prepared by an emulsion-solvent evaporation method using ethanol/liquid paraffin system. The resultant microspheres were evaluated for average particle size, drug loading, In-vitro drug release and release kinetics. FTIR spectrometry, scanning electron microscopy, differential scanning calorimetry and x-ray powder diffractometry were used to investigate the physical state of the drug in the microspheres.

19) Meral YUCE., ***et al*. (2008**) aim of this work was to investigate the influence of process variation in polymer type via viscosity grades of ethylcellulose N10 and N100, drug to polymer ratio, stirring rate of the propeller and surfactant type on the micromeritic properties of microspheres such as particle size distribution, bulk and tapped density, surface topography, tangent of angle of repose, compressibility index, Hausner ratio and flow rates. Ethyl cellulose N10 and N100 membrane materials indicated difference in release patterns of microspheres. Microspheres exhibited lower burst effect with decreased drug release rate, when the drug was incorporated with ethyl cellulose N100 and higher ratio of each polymer. Therefore, indomethacin release from ethyl cellulose microspheres could not be evaluated by any of the kinetic models.

20) V.V Prasanth., *et al*. (2008) were aimed to prepare Salbutamol sulphate (SS) loaded microspheres were prepared by solvent evaporation method with combination of hydroxy propyl methyl cellulose and Carbopol polymers in various proportions. A total of eleven formulations were prepared. It was confirmed with the results of micromeretic properties like swelling index particle size, entrapment efficiency property that all the selected formulations showed good flow property. Release data were analyzed based on Highuchi kinetics and

Korsmeyer-Peppas equation and all the selected formulations showed good fit to Highuchi model. Stability studies showed almost negligible changes in particle size, entrapment efficiency and drug release throughout the study period**.**

21) Sunit kumar sahoo., *et al*. (2005) Microspheres were prepared by solvent evaporation method using an acetone / liquid paraffin system. Magnesium stearate . The prepared microspheres were characterized for their micromeritic properties and drug loading, as well by fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry, x-ray powder diffractometry and scanning electron microscopy. The In-vitro release studies were performed in pH 6.8, phosphate buffer. The best-fit release kinetics was achieved with Higuchi plot followed by zero order and First order. The release of stavudine was influenced by the drug to polymer ratio and particle size & was found to be diffusion controlled.

22) Navneet Garud., *et al*. (2003) were prepared Metformin microspheres for prolonged release by non-aqueous solvent evaporation method using various polymers, including ethyl cellulose(EC), hydroxypropyl methyl cellulose (HPMC), carbopol 934P (CA) and chitosan (CH) .The effect of process variables ,viz, drug/polymer ratio, stirring rate and type of polymer on the mean particle size, drug entrapment efficiency, percentage yield, drug content, micromeritic properties and drug release of the microspheres were studied.

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2.2 DRUG PROFILE

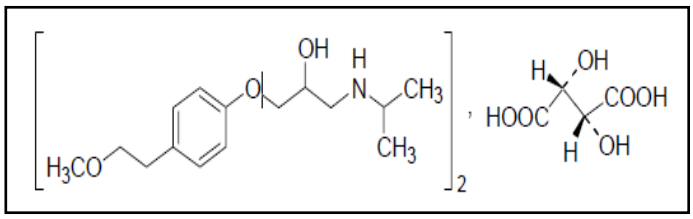
METOPROLOL TARTRATE:

(IP., 2007; Merck Index., 1997; USP., 2009; Tripathi K.D., 2004)

Metoprolol is the prototype of cardio selective (β1) blocker used in the treatment of several diseases cardiovascular system especially hypertension. It has the potency to block cardiac stimulation and it also induce vasodilatation. The active substance metoprolol is employed either as metoprolol succinate or metoprolol tartrate i.e 100 mg of metoprolol tartrate corresponds to 95 mg of metoprolol succinate. The tartrate is immediate release and the succinate is an extended release.

Chemical name:
(*RS)-1-isopropylamino-3-p- (2*-methoxyethyl) phenoxypropan-2-ol (2*R,3R)-tartrate*

Structural formula:



Category:

Adrenergic Agents, Adrenergic beta-Antagonists, Anti-Arrhythmia Agents Antihypertensive Agents, Sympatholytics

Dose:

Conventional dose: - Initially 50 to 100 mg daily in single or divided doses may increases weekly to 400 mg daily.

Maintenance dose: - 100 to 200 mg daily

Extended release preparation: - 25 to 100 mg once daily.

C. Physical properties:

Molecular formula : $C_{15}H_{25}NO_3)_2$, $C_4H_6O_6$

Molecular weight : 684.8 g/mol

Description : White powder or colorless crystals

Odour : Odour less

Taste : Metallic

Melting point : Melt at about 121 º to 124 ºC (BP)

Dissociation constant (pKa): 9.7

Solubility : Very soluble in water; freely soluble in ethanol; slightly soluble in acetone

Storage : Store in well closed container

D. Mechanism of action:

Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta (1)-adrenergic receptors in the heart and vascular smooth muscle. Beta (1)-receptor blockade results in a decrease in heart rate, cardiac output, blood pressure.

E. Pharmacokinetics:

Oral bioavailability : 40 to 50%

Protein binding : 12 %

Volume of distribution : 4 L/kg.

Half life : 3-7 hour

F. Absorption:

Metoprolol is readily and completely absorbed from the gastrointestinal tract, but is subjected to very considerable first pass metabolism in the liver and the bioavailability is only about 38%. Peak plasma concentrations vary widely and occur about 1.5 to 2 hrs after a single oral dose.

G. Distribution:

Metoprolol is widely distributed. It crosses the blood brain barrier the placenta and is distributed into breast milk. The apparent volume of distribution range from about 2.5 liters/kg to 5.0 liters/kg and approximately 90% bound to plasma protein.

H. Metabolism:

It is extensively metabolized in the liver by oxidative deamination, O-dealkylation followed by oxidation and aliphatic hydroxylation. Minimal hepatic metabolism by aldehyde oxidase, alcohol dehydrogenase, and aldehyde dehydrogenase to form inactive metabolites.

I. Excretion:

The metabolites are excreted in the urine together with only small amounts of unchanged metoprolol.

Therapeutic uses:

Hypertension,

Angina pectoris

Cardiac arrhythmias,

Myocardial infraction,

Migraine prophylaxis and Hyperthyroidism.

Drug interactions:

Beta-blocker and Calcium channel blocker have additive effect on the cardiac conducting system.

Toxicity:

LD_{50}=5500 mg/kg (orally in rats), toxic effects include bradycardia, hypotension, bronchospasm, and cardiac failure. LD_{50}=2090 mg/kg (orally in mice).

Dosage forms:

Table 2.1. Dosage forms and routes of administration of Metoprolol

Form	Route
Liquid	Intravenous
Solution	Intravenous
Film	Oral
Film extended release	Oral

Marketed preparations:

Beloc, Betaloc, Lopresor, Lopresoretic, Lopressor, Lopressor, HCT,Metroprolol, Prelis, Selo-Zok, Seloken, Selopral, Toprol, Toprol- XL

2.3. POLYMERS PROFILE:

2.3.1. EGG ALBUMIN

Synonyms : Ovalbumin, Egg white, Dried egg white

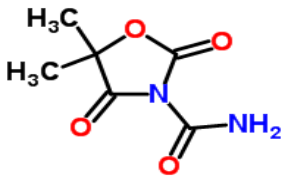
CAS registry number : 9006-50-2

Empirical formula : $C_6H_8N_2O_4$

Molecular weight : 44.3 Kda

Extinction Coefficient : $E^{1\%}$= 6.9-7.6(280nm) [1]

Structural formula :



Denaturation Point : Egg albumin denatures at 840^0C

Descriptions:

It is a phosphorylated- glycoprotein. The peptide portion of the molecule consists of 385 residues & has a molecular weight of 42.7 Kda.This sequence completely agrees with the reported mRNA sequence.

The carbohydrate & Phosphate portions account for an additional 1428 & 160 gm per mole respectively, giving a total molecular weight of 44.3 Kda.

Egg albumin in its structure has the following Amino acids.

20Lys, 10Tyr, 6Cys, 14 Asp & 33 Glu.

The above amino acids make egg albumin suitable for conjugation.

Typical properties:

Density **:** 430 kg/cm^3

Boiling point **:** 251.7 °C at 760 mm

Moisture content **:** contains 5 % to 8 % and not

Solubility **:** soluble in ethanol, soluble in water.

Surface tension **:** 12.5 % solution , pH 7.8, 24ºC at 49.9 dynes cm^{-1}

Stability and storage:

Egg albumin powder stable at room temperature . It should be stored in dry and clean, free from foreign smell, recommended storage temperature 8-26^oC;day fluctuations of temperature not more than4^oC.

Incompatibilities:

It is incompatible with other materials and strong oxidizing agent.

Handling precautions:

Observe normal precautions appropriate to the circumstances and quantity of material handled.

Regulatory statuses:

Included in the FDA Inactive Ingredients data base (oral tablets microspheres, film coating, and IV injections). Included in non parenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

2.3.2 ETHYL CELLULOSE

Nonproprietary Names:

BP : Ethyl cellulose

PhEur : Ethyl cellulose

USP-NF : Ethyl cellulose

Synonyms:

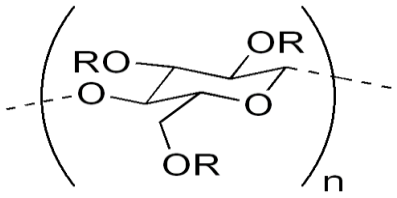
Aquacoat ECD, Aqualon, Ashacel, E462,Ethocel, ethylcellulosum Sure lease.

Chemical Name : Cellulose ethyl ether

CAS Registry Number: [9004-57-3]

Empirical Formula and Molecular Weight: Ethyl cellulose is partially ethoxylated. Ethyl cellulose with complete ethoxyl substitution (DS = 3) is $C_{12}H_{23}O_6$ ($C_{12}H_{22}O_5$) $nC_{12}H_{23}O_5$ where n can vary to provide a Wide variety of molecular weights. Ethyl cellulose, an ethyl ether of cellulose, is a long-chain polymer of b- anhydroglucose units joined together by acetal linkages.

Structural Formula:



R = H or CH_2CH_3

Functional Category:

Coating agent, flavoring agent, tablet binder, tablet filler, viscosity increasing agent.

Description:

Ethyl cellulose is a tasteless, free-flowing, and white to light tan-colored powder.

Color: white to light tan-colored powder

Odor **:** odorless.

Taste **:** tasteless

Texture **:** powder

Solubility:

Ethyl cellulose is practically insoluble in glycerin, propylene glycol, and water. Ethyl cellulose that contains less than 46.5% of ethoxyl groups is freely soluble in chloroform, methyl acetate, and tetrahydrofuran, and in mixtures of aromatic hydrocarbons with ethanol (95%). Ethyl cellulose that contains not less than 46.5% of ethoxyl groups is freely soluble in chloroform, ethanol (95%), ethyl acetate, methanol, and toluene.

Stability and Storage Conditions:

Ethyl cellulose is a stable, slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated, and to salt solutions, although it is more sensitive to acidic materials than are cellulose esters. Ethyl cellulose is subject to oxidative degradation in the presence of sunlight or UV light at elevated temperatures. This may be prevented by the use of antioxidant and chemical additives that absorb light in the 230–340nm range. Ethyl cellulose should be stored at a temperature not exceeding328°C (908F) in a dry area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.

Incompatibilities:

Incompatible with paraffin wax and microcrystalline wax.

Applications in Pharmaceutical Formulation or Technology

- Ethyl cellulose is widely used in oral and topical pharmaceutical formulations.
- The main use of ethyl cellulose in oral formulations is as a hydrophobic coating agent for tablets and granules. Ethyl cellulose coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the stability of a formulation. For example where granules are coated with ethyl cellulose to inhibit oxidation.
- Modified-release tablet formulations may also beProduced using ethyl cellulose as a matrix former. Ethyl cellulose, dissolved in an organic solvent or solvent mixture, can be used on its own to produce water-insoluble films.
- Drug release through ethyl cellulose-coated dosage forms can be controlled by diffusion through the film coating. This can be a slow process unless a large surface area (e.g. capsules or granules compared with tablets) is utilized. In those instances, aqueous ethyl cellulose dispersions are generally used to coat granules or capsules.
- Ethyl cellulose-coated beads and granules have also demonstrated the ability to absorb pressure and hence protect the coating from Fracture during compression.
- High-viscosity grades of ethyl cellulose are used in drug microencapsulation.
- Release of a drug from an ethyl cellulose microcapsule is a function of the microcapsule wall thickness and surface area.
- In tablet formulations, ethyl cellulose may additionally be employed as a binder, the ethyl cellulose being blended dry or wet granulated with a solvent such as ethanol (95%).

- Ethyl cellulose produces hard tablets with low friability, although they may demonstrate poor dissolution. Ethyl cellulose has also been used as an agent for delivering therapeutic agents from oral (e.g. dental) appliances.
- In topical formulations, ethyl cellulose is used as a thickening agent in creams, lotions, or gels, provided an appropriate solvent is used. Ethyl cellulose has been studied as a stabilizer for emulsions. Ethyl cellulose is additionally used in cosmetics and food products.

Table I: Uses of ethyl cellulose.

Use	Concentration (%)
Microencapsulation	10.0–20.0
Sustained-release tablet coating	3.0–20.0
Tablet coating	1.0–3.0
Tablet granulation	1.0–3.0

2.3.3. EUDRAGIT RL 100

Non-proprietary names:

Ph. Eur: Ammonio Methacrylate Copolymer, Type

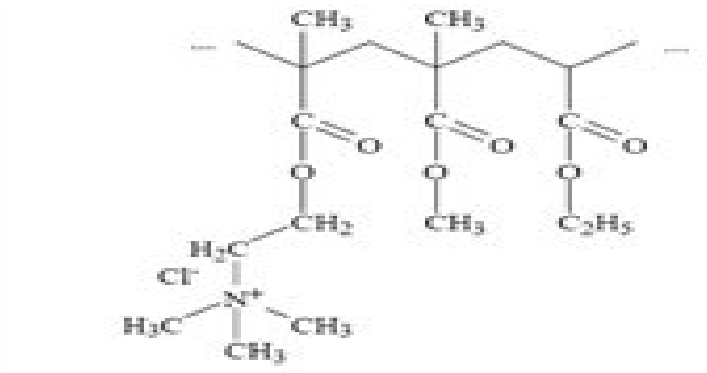
USP/NF: Ammonio Methacrylate Copolymer, Type A - NF

JPE: Aminoalkyl Methacrylate Copolymer RS

Synonyms **:** amino methacrylate copolymer

C.A.S. No. **:** 25086 – 15 – 1

Structure **:**



Chemical/IUPAC name **:** Poly (methacylic acid-co-methyl methacrylate) 1:2

INCI name **:** Acrylates Copolymer

Description **:** White powders with a faint characteristic odour.

Functional category:

Film former; tablet binder; tablet diluents.

Application in pharmaceutical formulation or technology:

EuagitR100 are used to form water insoluble film coating agent for sustained release products. Eudragit RL 100 films are more permeable than those

of eudragit RS, and films of varying permeability can be obtained by mixing the two types together.

Solubility:

1gm of Eudragit RL 100 dissolves in 7 g methanol, ethanol, in aqueous isopropyl alcohol ethanol, and acetone (containing approx.3% water),.

Film formation:

When the test solution is poured onto a glass plate, a clear film forms upon evaporation of the solvent.

Characteristics:

- Granulation of drug substances in powder form for controlled release
- Effective and stable enteric coatings with a fast dissolution in the upper Bowel
- Site specific drug delivery in intestine by combination with Eudragit S grades
- Variable release profiles

Applications:

Eudragit is widely used in oral pharmaceutical formulations, especially in targeting drug delivery system in GIT. Eudragit is used to prepare sustained-release preparations.

Loss on Drying: Max. 5.0 % according to "Dry substance / Residue on evaporation."

Viscosity (dynamic): 50 - 200 mPas.

Stability and Storage:

Minimum stability dates are given on the product labels and batch-related Certificates of Analysis. Protect from warm temperatures (USP, General Notices). Protect against moisture.

AIM & OBJECTIVES....

3. AIM AND OBJECTIVES

Metoprolol tartrate is a competitive β1- selective adrenergic antagonist. It is widely used for the treatment of hyper tension, angina pectoris and prophylaxis of migraine receptors. This drug is water soluble although it is well absorbed in the gastro intestinal tract and its bio availability is low (40 to 50%). It has a short biological half life (3 to 4 hours) and is usually administered in a dose of 25 to100 mg two times daily in order to maintain effective concentrations throughout the day. In long -term therapy, fluctuation in the plasma concentration, with high concentration peaks are common for drugs with rapid absorption and elimination if plasma levels markedly exceed therapeutic levels. Such characteristics make Metoprolol tartrate as best suitable drug candidates for controlled drug delivery.

Microsphere formulation offers a number of advantages in therapeutics where the controlled release of drugs as well as predictable and reproducible drug release kinetics is important factors among them. Metoprolol tartrate is the most widely used anti- hypertensive agent.

The aim of the present work was to formulate Metoprolol Tartrate microspheres using natural, semi synthetic and synthetic polymers containing in order to provide a controlled release effect and relatively constant effective level of these drugs in the treatment of major Hypertension disease.

The main objective of the present investigation was to develop and to evaluate the Metoprolol tartrate microspheres by using natural polymer-Egg albumin; semi synthetic polymer-Ethyl cellulose and synthetic polymers-Eudragit RL 100.The effect of variable concentration of polymers on the characteristics of the microspheres

will be studied. Since these polymers are biocompatible it has been reported in reputed journals.

Since nine formulations were prepared with varying drug and polymer ratio. These formulations were subjected to evaluate the various parameters like percentage yield, drug content, entrapment efficiency, particle size, FTIR, DSC, SEM, *In-vitro* drug release, Kinetic drug release and stability studies.

PLAN OF WORK

4. PLAN OF WORK

LITERATURE SURVEY

SELECTION OF DRUG AND POLYMERS

PROCUREMENT OF DRUG AND POLYMERS

EXPERIMENTAL WORK

Preformulation Study

Identification of Drug

- Organoleptic properties of drug
- Melting point
- Solubility profile
- UV-Spectroscopy (λ_{max})
- Quantification of drug
- FTIR Spectroscopy
- Loss on drying

- **Formulation of Metoprolol Tartrate Microspheres**
- **Evaluation of Microspheres**
  - Percentage Yield
  - Estimation of drug content
  - Estimation of drug entrapment efficiency
  - Particle size analysis
  - Differential scanning calorimetric (DSC)
  - Scanning electron microscopy (SEM)

- *In-vitro* drug release study
- Kinetics of *in-vitro* drug release
- Stability study

RESULTS AND DISCUSSION

SUMMARY AND CONCLUSION

FUTURE PROSPECTS

BIBLIOGRAPHY

MATERIALS &

EQUIPMENTS....

5. MATERIALS AND EQUIPMENTS

5.1. List of Materials used with Sources

Table 5.1: List of Polymers and Excipients with source

S.No.	Ingredients	Supplier
1	Metoprolol Tartrate	Madras pharmaceuticals pvt Ltd, Chennai.
2	Egg albumin	Dr. Reddys pharmaceuticals limited, Mumbai.
3	Ethyl cellulose	S d fine-chem limited, Mumbai.
4	Eudragit RL 100	Hi-media laboratories, Mumbai.
5	Coconut oil	marico. limited, Mumbai.
6	Light liquid paraffin	Qualigens fine chemicals, Mumbai.
7	Sodium lauryl sulphate	S d fine-chem limited, Mumbai.
8	Tween 80	Fischer scientific chemicals, Mumbai.
9	Acetone	Fischer scientific chemicals, Mumbai.
10	Ethanol	Fischer scientific chemicals, Mumbai.
11	n-Hexane	Loba Chemicals Pvt.Ltd. Mumbai.
12	Hydrochloric acid	S d fine-chem limited, Mumbai.

5.2. List of Equipments used with model:

Table 5.2: List of Equipments with model/make

S.No	Equipments	Model/ Make
1	Electronic balance	Shimadzu BL-220H.
2	Mechanical stirrer	2200MH, Soltech srl, Soluzioni Tecnologirhe, Milano, Italy.
3	Magnetic stirrer	Labtech, Ambala.
4	Digital pH meter	Elico scientifics-L1610, Mumbai.
5	UV spectrophotometer	Shimadzu-1700 Pharmaspec UV- VISIBLE
6	FTIR spectrophotometer	Shimadzu S4008.
7	Differential scanning calorimeter	Shimadzu DSC 60 with DTA, Japan.
8	Scanning electron microscope	SEM, JSM-6360LV Tokyo, Japan.
9	Humidity chamber	Lab tech.
10	USP Tablet Dissolution Apparatus Type II	USP XXIV (Electro Lab, Mumbai).

PRE-FORMULATION STUDIES....

6. PRE-FORMULATION STUDIES

Preformulation Study: *(Jens T., 2000)*

Before the formulation of a product should be investigation of physical and chemical properties of a drug substance alone to effective, stable and safe dosage form. It is the first step in rational development of dosage form.

6.1. Identification of Drug:

The preliminary studies were carried out by testing of different physical and chemical properties of drug as follows.

6.1.1. Organoleptic Properties of Drug: *(Lachman L., 1991)*

The Organoleptic properties like physical state, color, taste, odor etc., of the drug was reported with help of the descriptive terminology. It helps to identify the drug.

6.1.2. Melting Point:

It is the easy way to identify the drug. The melting point of Metoprolol tartrate was tested by use of a laboratory melting point apparatus with capillary tube method a procedure given in official monographs.

6.1.3. Solubility Profile: *(IP., 2007)*

It is important to know about solubility characteristic of a drug in aqueous system, since they must possess some limited aqueous solubility to elicit a therapeutic response. The solubility of drug was recorded by using various descriptive terminology specified in Indian pharmacopoeia, 2007.

Table 6.1: Description of solubility

Descriptive term	Parts of solvent required for 1 part of solute
Very soluble	Less than1
Freely soluble	From 1 to10
Soluble	From 10 to30
Sparingly soluble	From 30 to100
Slightly soluble	From 100 to 1,000
Very slightly soluble	From 1,000 to 10,000
Practically insoluble	Greater than or equal to 10,000

6.1.4. UV Spectroscopy (λ max): *(Venkatalakshmi Ranganathan., 2011)*

The absorption maximum of the standard solution was scanned between200- 400 nm regions on Shimadzu-1700 Pharmaspec UV-visible spectrophotometer. The absorption maximum obtained with the substance being examined corresponds in position and relative intensity to those in the reference spectrum.

6.1.4.1. Development of Standard Curve of Metoprolol tartrate in Distilled Water:

Preparation of Stock Solution of Metoprolol tartrate in Distilled Water:

Weighed accurately about 100 mg of Metoprolol tartrate was dissolved in little quantity of distilled water and volume was adjusted to 100 ml with the same to prepare standard solution having concentration of 1000 µg/ml. From this solution, pipette out 10 ml and made up to 100 ml with distilled water to produce 100 µg/ml.

Procedure:

From the stock solution, aliquots of 1, 2, 3, 4 and 5 ml were transferred to 10 ml volumetric flasks and final volume was made to 10 ml with distilled water to get 10 to 50 µg/ml. Absorbance values of these solutions were measured against blank (Distilled water) at 274.5 nm using UV-visible spectrophotometer.

6.1.4.2. Development of Standard Curve of Metoprolol tartrate in 0.1N HCl:

Preparation of 0.1N HCl:

0.1N HCl was prepared according to I.P. 1996. Accurately 8.5 ml of HCl was taken and diluted with freshly prepared distilled water to produce 1000 ml.

Preparation of Stock Solution of Metoprolol tartrate in 0.1 N HCl:

Weighed accurately about 100 mg of Metoprolol tartrate was dissolved in little quantity of 0.1N HCl and volume was adjusted to 100 ml with the same to prepare standard solution having concentration of 1000 µg/ml. From this solution, pipette out 10 ml and made up to 100 ml with 0.1N HCl to produce 100µg/ml.

Procedure:

From the stock solution, aliquots of 1, 2, 3, 4 and 5 ml were transferred to 10 ml volumetric flasks and final volume was made to 10 ml with 0.1N HCl to get 10 to 50 µg/ml. Absorbance values of these solutions were measured against blank 0.1 N HCl at 274.5 nm using UV-visible spectrophotometer.

6.1.4.3. Development of Standard Curve of Metoprolol tartrate in pH 7.4 Phosphate Buffer:

Preparation of 0.2M Potassium Dihydrogen phosphate:

Dissolved 27.218 gm of potassium dihydrogen phosphate in water and made up to 1000 ml.

Preparation of 0.2M Sodium hydroxide:

Dissolved 8 gm of sodium hydroxide pellets in distilled water and made up to 1000 ml.

Preparation of Phosphate Buffer pH 7.4:

Phosphate buffer pH 7.4 was prepared according to I.P. 2007. A quantity of 50 ml of 0.2M potassium dihydrogen phosphate and 39.1 ml of 0.2M sodium hydroxide was diluted with freshly prepared distilled water to produce 200 ml.

Preparation of Stock Solution of Metoprolol tartrate in pH 7.4 Phosphate Buffer:

Weighed accurately about 100 mg of Metoprolol tartrate was dissolved in little quantity of pH 7.4 phosphate buffer and volume was adjusted to 100 ml with the same to prepare standard solution having concentration of 1000 µg/ml. From this solution, pipette out 10 ml and made up to 100 ml with pH 7.4 phosphate buffer to produce 100 µg/ml.

Procedure:

From the stock solution, aliquots of 1, 2, 3, 4 and 5 ml were transferred to 10 ml volumetric flasks and final volume was made to 10 ml with pH 7.4 phosphate buffer to get10 to 50 µg/ml. Absorbance values of these solutions were measured against blank (Phosphate Buffer pH 7.4) at 274.5 nm using UV-visible spectrophotometer.

6.1.5. Quantification of Drug:

Accurately weighed 100 mg of Metoprolol tartrate was dissolved in little quantity of 0.1 N HCL and volume was adjusted to 100 ml with the same to prepare standard solution having concentration of 1000 µg/ml. From this solution, pipette out10 ml and made up to 100 ml with 0.1NHCL to produce 100µg/ml. From the above solution, aliquots of 2 ml were transferred to 10 ml volumetric flasks and final volume was made to 10 ml with 0.1 N HCL. Absorbance values of these solutions were measured against blank 0.1N HCL at 274.5 nm using Shimadzu-1700 Pharmaspec UV-visible spectrophotometer. The percentage purity of drug was calculated by using calibration graph method (least square method).

Fourier Transforms Infra-Red (FTIR) Spectroscopy:

(Robert M. Silverstein, 2003; Becket A. H. and Stenlake J. B., 2005)

FTIR study was carried out to check identity of drug. Infrared spectrum of Metoprolol tartrate was determined on Fourier transform Infrared Spectrophotometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run followed by drug by using FTIR spectrophotometer. The absorption maximums in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum.

Loss on drying: *(Indian Pharmacopoeia, 2007)*

Loss on drying is the loss of weight expressed as percentage w/w resulting from volatile matter of any kind that can be driven off under specified condition. The test can be carried out on the well mixed sample of the substance.

$$\text{Loss on drying} = \frac{\text{Initial weight of substance} - \text{Final weight of substance}}{\text{Initial weight of substance}} \times 100$$

FORMULATION OF MICROSPHERES....

7. FORMULATION OF MICROSPHERES

Table.7.1: Formulation of Metoprolol Tartrate Microspheres

S. NO	FOMULATION CODE	METOPROLOL TARTRATE(gm)	EGG ALBUMIN(gm)	ETHYL CELLULOSE(gm)	EUDRAGIT RL 100 (gm)
1	F1	1	1	-	-
2	F2	1	2	-	-
3	F3	1	3	-	-
4	F4	1	-	1	-
5	F5	1	-	2	-
6	F6	1	-	3	-
7	F7	1	-	-	1
8	F8	1	-	-	2
9	F9	1	-	-	3

Formulations F1, F2, and F3 – Metoprolol Tartrate + Egg albumin

Formulations F4, F5, and F6 – Metoprolol Tartrate + Ethyl cellulose

Formulations F7, F8, and F9 – Metoprolol Tartrate +Eudragit RL 100

.

METHODS OF PREPARING MICROSPHERES:

The following are the methods used for the preparation of Microspheres. They are as follows

- Emulsion polymerization method.
- Solvent evaporation method

PREPARATION OF EGG ALBUMIN MICROSPHERES BY PHASE SEPARATION AND EMULSION POLYMERIZATION METHOD:

Phase separation emulsion polymerization method was employed for the preparation of microspheres. Polymeric drug solution was prepared by adding 1gm of drug (Metoprolol tartrate) to 10ml of 10% solution of Egg albumin and stirred continuously until uniform dispersion. In a separate beaker, to 86ml of coconut oil 1ml of 0.5% sodium lauryl sulphate was added drop-wise using 22 gauge needle into the organic phase and stirred continuously to form uniform dispersion. The temperature of the solution was gradually increased to 80°C and stirred at this temperature for 2hours. The solution was cooled to room temperature was attained, 1ml of formaldehyde and 20 ml of n-Hexane then followed by distilled water, dried and stored in air tight container until further analysis.

Fig.7.1: Preparation of egg albumin microspheres

PREPARATION OF ETHYL CELLULOSE MICROSPHERES BY SOLVENT EVAPORATION METHOD:

Solvent evaporation method was employed for the preparation of microspheres, containing 1gm of ethyl cellulose was dissolved in 20ml of acetone and ethanol solution in the ratio (1:1).To the above polymeric solution, 1gm of metoprolol tartrate was added and dispersed. The resultant mixture was added drop-wise using 22 gauge needle into 100ml oil phase containing 50 ml of coconut oil and 50 ml of light liquid paraffin containing 0.2ml of Tween 80. The resultant dispersion was stirred continuously using mechanical stirrer at 400 rpm for 3-4 hours**.** Then finally the microspheres were collected and washed with n-Hexane to remove the oil content followed by washing with distilled water thrice .The microspheres were then filtered, dried and stored in air tight container until further analysis. During the preparation procedure, emphasis is to be given on rpm and temperature for size and spherecity of the microspheres.

PREPARATION OF EUDRAGIT RL 100 MICROSPHERES BY SOLVENT EVAPORATION METHOD:

Solvent evaporation method was employed for the preparation of microspheres, containing 1gm of Eudragit RL 100 was dissolved in 20ml of acetone and ethanol solution in the ratio (1:1).To the above polymeric solution, 1gm of metoprolol tartrate was added and dispersed. The resultant mixture was added drop-wise using 22 gauge needle into 100ml oil phase containing 50 ml of coconut oil and 50 ml of light liquid paraffin containing 0.2ml of Tween 80. The resultant dispersion was stirred continuously using mechanical stirrer at 1500 rpm for 8 hours**.** Then finally the microspheres were collected and washed with n-Hexane to remove the oil

content followed by washing with distilled water thrice .The microspheres were then filtered, dried and stored in air tight container until further analysis. During the preparation procedure, emphasis is to be given on rpm and temperature for size and spherecity of the microspheres.

Fig.7.2: Preparation of Eudragit RL 100 microspheres

EVALUATION OF MICROSPHERES....

8. EVALUATION OF MICROSPHERES

8. EVALUATION OF MICROSPHERES

8.1. Percentage Yield: ***(****Venkatesan P et al., 2011; Bindhu Madhavi., et al., 2011)*

The dried microspheres were weighed and percentage yield of the prepared microspheres was calculated by using the following formula.

Percentage yield = (Weight of Microspheres/Weight of Polymer + drug) X 100

8.2. Estimation of Drug content: ***(****Chinna gangadhar et al., 2010)*

The prepared microspheres were powdered and passed through sieve no (85/120). The powder retained on the sieve 120 was taken for content uniformity studies. A weight of powder containing 100 mg of the drug was taken in a 100ml standard volumetric flask. To this of 0.1 N HCl solutions was added and made up to the mark with 0.1 N HCl solution and kept overnight. The final solution was filtered using what man filter paper. From this 10 ml was pipette out into a 100 ml standard volumetric flask and made up to the volume with 0.1 N HCl solution and estimated.

$$\textbf{Percentage drug content} = \frac{\textbf{Amount of drug found}}{\textbf{Label claim}} \times 100$$

8.3. Estimation of Entrapment efficiency: ***(****Chinna gangadhar et al., 2010)*

To evaluate the amount of the drug inside the microspheres, an indirect method was used. Aliquots from the filtered solutions remaining after removal of the microspheres were assayed spectrophotometrically. The amount of drug entrapped was calculated from the difference between the total amount of drug added and the amount of drug found in the filtered solution. About 100 mg of microspheres were completely dissolved in 500 ml of phosphate buffer solutions (pH 7.4), and stirred for

1h. Then, 2 ml of solution was filtered and the concentration of drug was determined spectrophotometrically by UV. Efficiency of drug entrapment was calculated in terms of percentage drug entrapment (PDE) as per the following formula

$$\textbf{Percentage drug entrapment efficiency} = \frac{\textbf{W initial drug} - \textbf{W free drug}}{\textbf{W initial drug}} \times 100$$

8.4. Particle size Analysis: *(A.R. Shabarya., et al.,2003)*

Microspheres were dispersed in liquid paraffin and observed under 200 x magnification using an optical microscope. The size distribution was carried out by optical microscopy. An average of about 100 particles were counted and determined.

8.5. Differential Scanning Calorimetry Study (DSC): *(Jain N. K., 2008)*

Metoprolol tartrate powder was mixed with various polymers in the ratio of 1:1. The mixture of drug with polymers to maximize the like hood of obscuring an interaction. Mixture should be examined under Nitrogen to eliminate oxidative and pyrolytic effect at a standard heating rate (10 $^{\circ}$C/minute) on DSC. Over a temperature range, this will encompass any thermal changes due to the mixture of drug with polymers. Thermograms of pure drug are used as a reference.

Appearance or disappearances of one or more peaks in thermograms of drug with polymer are considered as a indication of interaction.

8.6. Scanning electron microscopy (SEM) (*Chinna Gangadhar., et al.,2010)*

The samples for the SEM analysis were prepared by sprinkling the microspheres on one side of a double adhesive stub. The stub was then coated with gold. The microspheres were then observed with the scanning electron microscope (SEM, JSM-6360LV scanning electron microscope, Tokyo, Japan) at 15kv. The samples include blank microspheres, drug loaded microspheres.

8.7. In-vitro Release Study ***(Karthikeyan., et al.,2003)***

In vitro drug release from microspheres was performed using the rotating basket method as specified in USP XXIV (Electro Lab, Mumbai). Microspheres equivalent to 100 mg of Metoprolol tartrate was placed into basket (tied using- muslin cloth) immersed in 900 ml of 0.1 N HCl (pH 1.2) dissolution medium and allowed to rotated at 100 rpm. Operating temperature was maintained at $37^{o} \pm 1^{o}C$. Sample (5ml) was withdrawn at predetermined time hour intervals up to 2hr. same volume was replaced to maintain sink condition. After 2hr, dissolution medium was changed by 900ml of fresh phosphate buffer of pH 7.4 and study was continued upto 12^{th} hour. The samples were analyzed by UV-spectrophotometer at 274.5 nm.

8.8. Kinetic of *In-vitro* drug release *(Mohan raj Palanisamy., et al., 2009)*

Five kinetic models including the zero order (Equation 1), first order (Equation 2), Higuchi matrix (Equation 3) and Peppas-Korsmeyer (Equation 4) release equations were applied to process the In-vitro released data to find the equation with the best fit using Microsoft Office Excel 2007.

$$R = k_1 t \quad \text{----------->} \quad \text{Equation 1}$$

$$\log UR = \frac{k_2 t}{2.303} \quad \text{----------->} \quad \text{Equation 2}$$

$$R = k_3 t^{0.5} \quad \text{----------->} \quad \text{Equation 3}$$

$$R = k_4 t^n \text{ or } \log R = \log k_4 + n \log t \quad \text{-------->} \quad \text{Equation 4}$$

Where R and UR are the released and unreleased percentages, respectively, at time (t); k1, k2, k3 and k4 are the rate constants of zero-order, first-order, Higuchi matrix, Peppas-Korsmeyer respectively.

Table 8.1: Diffusion exponent and solute release mechanism

Diffusion exponent (n)	**Overall solute diffusion mechanism**
< 0.5	Quasi-Fickian diffusion
0.5	Fickian diffusion
0.5 < n < 1.0	Anomalous (non-Fickian) diffusion
1.0	Case-II transport
> 1.0	Super case-II transport

8.8. STABILITY STUDY *(Manavalan R. and Ramasamy S., 2004)*

In any rational drug design or evaluation of dosage forms for drugs, the stability of the active component must be a major criterion in determining their acceptance or rejection. Stability of a drug can be defined as the time from the date of manufacture and the packaging of the formulation, until its chemical or biological activity was not less than a predetermined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously.

- **Objective of the study**

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enabling recommended storage conditions, re-test periods and shelf-lives. Generally, the observation of the rate at which the product degrades under normal room temperature

requires a long time. To avoid this undesirable delay, the principles of accelerated stability studies are adopted. The International Conference on Harmonization (ICH) Guidelines titled Stability testing of New Drug Substances and Products describes the stability test requirements for drug registration application in the European Union, Japan and the States of America.

ICH specifies the length of study and storage conditions

- **Long-Term Testing:** 2° C ±2° C at 60% RH ±5% for 12 Months

- **Accelerated Testing:** 40° C ±2° C at 75% RH ±5% for 6 Months

The selected formulation F3 was exposured up to 3 months of stability studies at accelerated condition (40° C ±2° C at 75% RH ± 5%RH) to find out the effect of percentage yield, drug content, entrapment efficiency and *In-vitro* drug release.

Procedure:

Stability studies were carried out at 40° C / 75% RH for the optimized formulation for 3 months. The microspheres were stored at 40° C/75% RH as per ICH guidelines and various parameters (drug content and drug release profile) were monitored periodically for 3 months.

RESULTS & DISCUSSION....

9. RESULTS AND DISCUSSION

9.1. Identification of Drug:-

9.1.1. Organoleptic Properties:

Colour : White

Odour : Odorless

Taste : Metallic

Appearance: Fine powder

9.1.2. Melting Point:

Melting point values of Metoprolol tartrate sample was found to be in range of 136.4^{0}C. The official melting point range is between136-138°C. Hence, results were complied the limits specified in official monographs.

9.1.3. Solubility Study:

Table 9.1: The solubility of Metoprolol tartrate in different solvents

Name of solvent	Parts of solvent required per part of solute	Solubility
Distilled water	10	Very soluble
Ethanol (95%)	40	Freely soluble
Chloroform	400	Sparingly soluble
Ether	600	Practically insoluble
0.1 N HCl	10	Very soluble
Phosphate buffer pH7.4	70	Freely soluble

9.1.4.1. UV Spectroscopy (λ max):

9.1.4.1. Determination of λ_{max} of Metoprolol tartrate by using Distilled Water

The absorption maximum for Metoprolol tartrate in Distilled Water was found to be 274.5nm and it is shown in Figure 9.1.

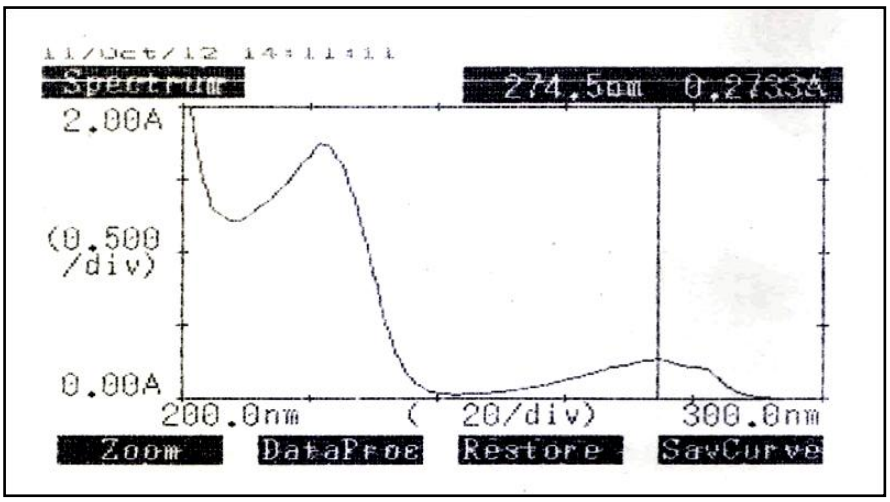


Fig. 9.1: λ_{max} Observed for Metoprolol tartrate in Distilled Water

9.1.4.2. Preparation of Standard Curve of Metoprolol tartrate by using Distilled Water:

UV absorption spectrum of Metoprolol tartrate in Distilled Water showed λ max at 274.5 nm. Absorbance obtained for various concentrations of Metoprolol tartrate in distilled water are given in Table 9.2. The graph of absorbance vs. concentration for Metoprolol tartrate was found to be linear in the concentration range of 10 - 50 µg/ml. The drug obeys Beer - Lambert's law in the range of 10-60µg/ml which is shown in Figure 9.2.

Table 9.2: Data of concentration and absorbance for Metoprolol tartrate for Distilled Water

S. No.	Concentration (µg/ml)	Absorbance at 274.5nm
1.	0	0
2.	4	0.140
3.	8	0.272
4.	12	0.396
5.	16	0.534
6.	20	0.681

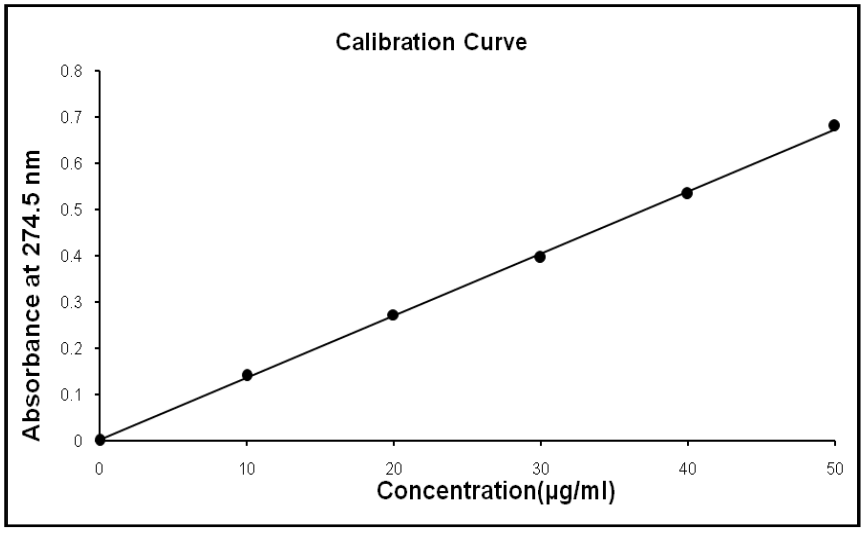


Fig. 9.2: Standard graph of Metoprolol tartrate in Distilled Water

The values of Correlation coefficient (R), Slope, Intercept obtained from the calibration curve are given in the Table 9.3.

Table 9.3: Data for calibration curve parameters in Distilled Water

S. No.	Parameters	Values
1.	Correlation Coefficient (r)	0.9999
2.	Slope (m)	0.0134
3.	Intercept (c)	0.0006

9.1.4.3. Determination of λ_{max} of Metoprolol tartrate by using 0.1N HCl:

The absorption maximum for Metoprolol tartrate in 0.1N HCl was found to be 274.5 nm and it is shown in Figure 9.3

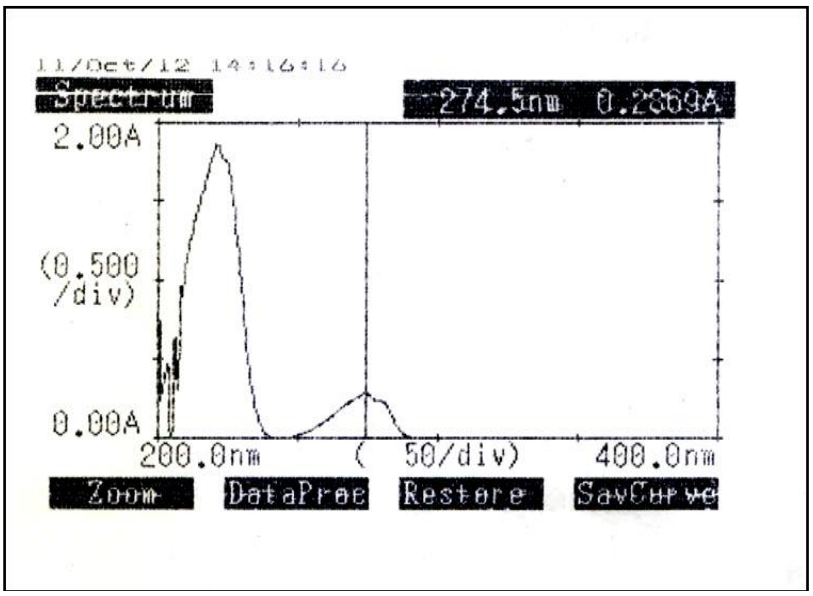


Fig 9.3: λ_{max} Observed for Metoprolol tartrate in 0 .1 N HCl

9.1.4.4. Preparation of Standard Curve of Metoprolol tartrate by using 0.1N HCl:

The UV absorption spectrum of Metoprolol tartrate in 0.1N HCl shows λ_{max} at 274.5 nm. Absorbance obtained for various concentrations of Metoprolol tartrate in 0.1 N HCl are given in Table 9.4. The graph of absorbance vs. concentration for Metoprolol tartrate was found to be linear in the concentration range of 10-50µg /ml. The drug obeys Beer - Lambert's law in the range of 10-50 µg /ml. This is shown in Figure 9.4 the calibration parameters were shown in Table 9.5.

Table 9.4: Data of concentration and absorbance for Metoprolol tartrate for 0.1N HCl

S. No.	Concentration (µg/ml)	Absorbance at 274.5 nm
1.	0	0.000
2.	10	0.136
3.	20	0.276
4.	30	0.419
5.	40	0.557
6.	50	0.690

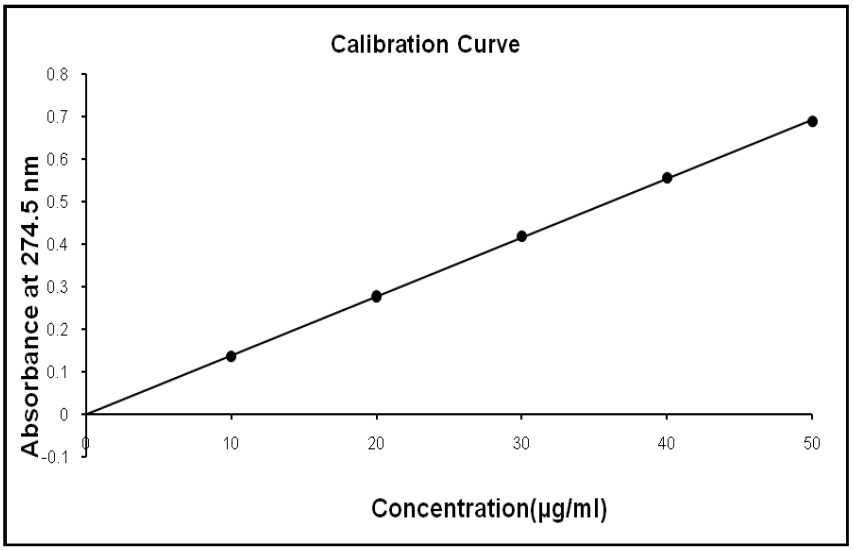


Fig.9.4 : Standard graph of Metoprolol tartrate 0.1N HCl

The values of Correlation coefficient (R), Slope, Intercept obtained from the calibration curve are given in the Table 9.5.

Table 9.5: Data for calibration curve parameters in 0.1N HCl

S. No.	Parameters	Values
1.	Correlation Coefficient (r)	0.9999
2.	Slope (m)	0.0138
3.	Intercept (c)	0.0003

9.1.4.5. Determination of λ_{max} of Metoprolol tartrate by using pH 7.4 Phosphate buffer:

The absorption maximum for Metoprolol tartrate in pH phosphate buffer was found to be 274.5 nm and it is shown in Figure 9.5

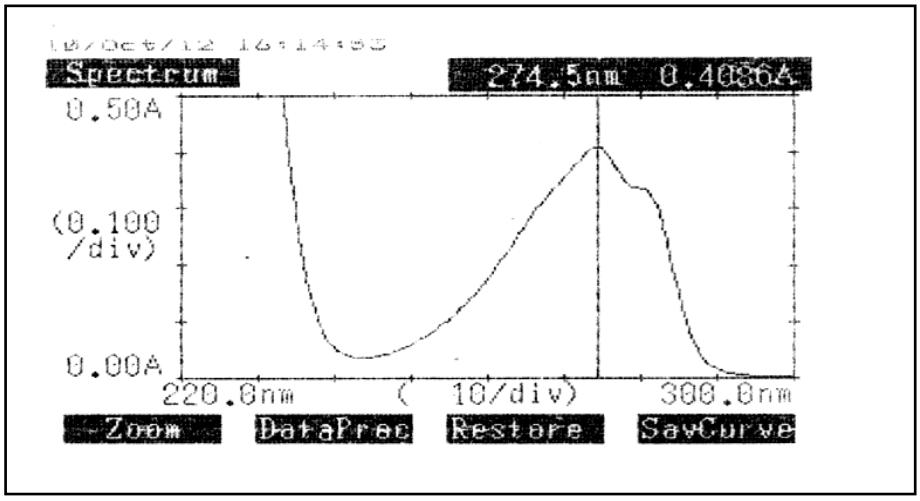


Fig 9.5: λ_{max} Observed for Metoprolol tartrate in Phosphate buffer pH 7.4

9.1.4.6. Preparation of standard curve of Metoprolol tartrate by using pH 7.4 Phosphate Buffer:

UV absorption spectrum of Metoprolol tartrate pH 7.4 phosphate buffer shows λ_{max} at 274.5 nm. Absorbance obtained for various concentrations of Metoprolol tartrate in pH phosphate Buffer are given in table 9.6. The graph absorbance vs concentration for Metoprolol tartrate was found to be linear in the concentration range of 10-50 µg /ml. The drug obeys Beer- Lambert's law in the range of 10-50 µg /ml which is shown in Figure 9.7

Table 9.6 : Data of concentration and Absorbance data for Metoprolol tartrate in pH 7.4 Phosphate Buffer

S. No.	Concentration (µg/ml)	Absorbance at 274.5 nm
1	0	0
2	10	0.132
3	20	0.263
4	30	0.401
5	40	0.526
6	50	0.657

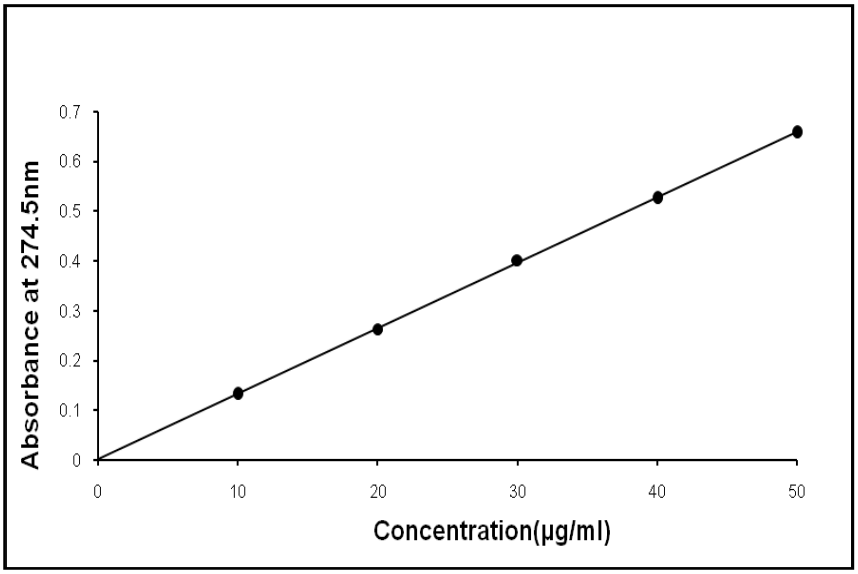


Fig. 9.6: Standard graph of Metoprolol tartrate pH 7.4 Phosphate buffer

Table 9.7: Data for Calibration Curve parameters in pH 7.4 Phosphate Buffer

The values of Correlation coefficient (R), Slope, Intercept obtained from the calibration curve are given in the following table 9.7.

S. No.	Parameters	Values
1	Slope	0.0131
2	Intercept	0.0006
3	Correlation coefficient (R)	0.9996

9.1.5. Quantification of Drug:

The percentage purity of drug was calculated by using calibration graph method (least square method) and the data has been shown in table 9.8.

Table 9.8: Percentage purity of Metoprolol tartrate

S. No.	Percentage Purity (%)	Average Percentage Purity* (%)
1.	99.44	99.67 ± 0.17
2.	99.71	
3.	99.83	

*All the values are expressed as mean ± SD, n = 3

The percentage purity for Metoprolol tartrate in IP 2007 is not less than 90.0 % and not more than 101.0 % of the stated amount of Metoprolol tartrate. The percentage purity of Metoprolol tartrate was found to be 99.67 ± 0.17. So, it stands within the limits of IP 2007

9.1.6. Fourier Transform Infra-Red Spectroscopy (FTIR):

The FTIR spectrum of Metoprolol tartrate shown in figure 9.7. The Interpretation of IR frequencies are shown in Table 9.9.

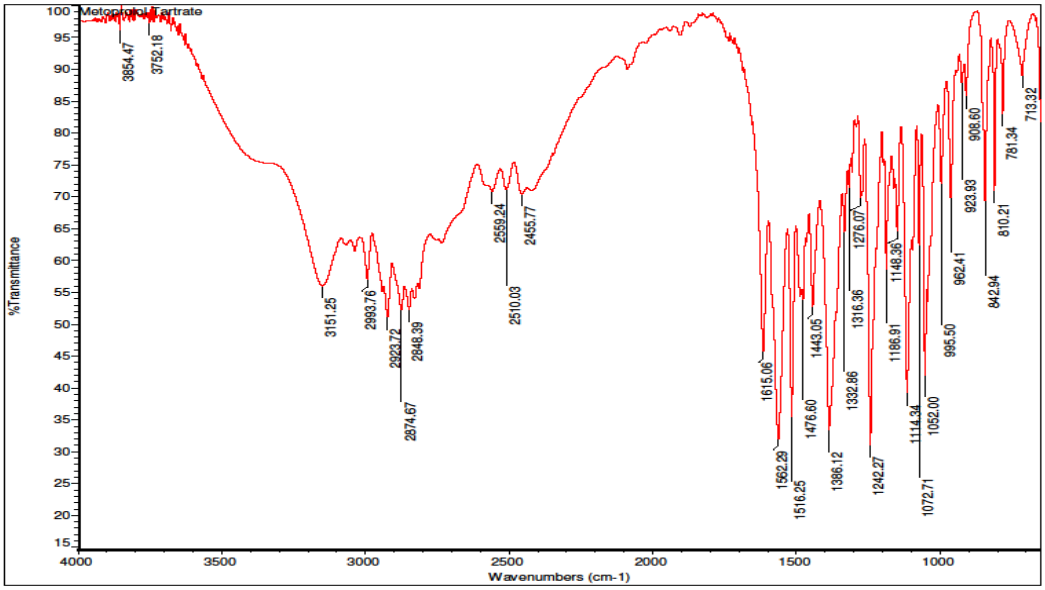


Fig. 9.7: FTIR Spectrum of Metoprolol Tartrate

Table 9.9: Interpretation of FTIR spectra of Metoprolol tartrate

O-H Stretching	3752.18
C-O-C Stretching Aliphatic	1114.34
C- N Stretching	1242.27
N-H bend Aliphatic	1615.06
N-H Stretch 2 Amine	3151.25

From the above figure 9.9, it can be seen that, the major functional group peaks observed in spectras of Drug with all the polymers remains unchanged as compared with spectra of Metoprolol tartrate. So fom the above FTIR spectra it can be observed that the identified as Metoprolol tartrate.

9.1.7. Loss on drying

The percentage loss on drying after 5 hours was found to be 0.208±0.003%. The sample passes test for loss on drying as per the limits specified in IP.

Table 9.10: Loss on drying of Metoprolol tartrate

S. No.	Percentage Loss on drying (%)	Average LOD (%)
1	0.205	0.208±0.003
2	0.206	
3	0.214	

All the values are expressed as a mean ± SD., n = 3

9.2. EVALUATION OF MICROSPHERES

9.2.1. Percentage Yield, Drug Content and Entrapment Efficiency

The percentage yield, Drug Content and Entrapment Efficiency of Controlled release microspheres were found to increased as the polymer ratio was increased. The maximum yield of microspheres was 87.16% in Egg albumin polymer, 80.48% in Ethyl cellulose and 83.48% in Eudragit RL 100. Better yield of microspheres was obtained from Egg albumin. Drug content and Entrapment efficiency was high in Egg albumin containing formulations when compared to Ethyl cellulose and Eudragit RL 100 formulations. All the formulations Percentage Yield, Drug content and Entrapment efficiency data was showed in Table 9.11.

Table 9.11: Comparison of Percentage yield, Drug content and Percentage Entrapment efficiency

S. No.	FORMULATIONS	% YIELD	% DRUG CONTENT	% ENTRAPMENT
1	F1	84.76	53.35±0.94	83.08±1.62
2	F2	87.90	56.81±1.31	85.12±1.21
3	F3	90.37	63.61±1.71	88.40±1.08
4	F4	78.38	36.76±1.59	72.49±1.20
5	F5	82.53	38.09±1.57	76.04±1.23
6	F6	86.46	46.15±1.50	79.42±0.41
7	F7	81.61	45.01±1.36	74.84±1.46
8	F8	83.17	47.92±1.81	77.89±0.36
9	F9	86.98	51.08±1.25	81.79±1.32

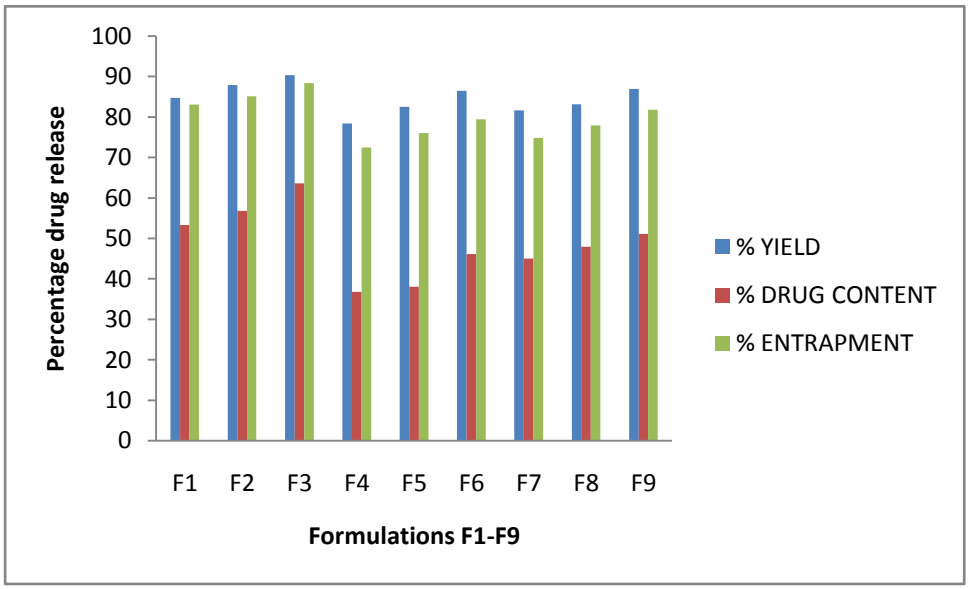


Figure 9.8: Percentage Yield, Drug Content and Entrapment Efficiency

9.2.2. Particle size analysis

Particle size of prepared microspheres was determined by optical microscopy method and the average particle sizes of all batches of microspheres were represented in Table. 9.12.

All the batches of microspheres were prepared by keeping the drug amount and solvent volume constant. The particle sizes of the sustained release microspheres were found to be in the range of 50±0.05 µm – 335±0.32 µm. The results were represented graphically in Figure 9.9.

Table 9.12: particle size for various formulations of microspheres

S.NO	Formulation Code	Particle size (µm±S.D)
1	F1	50±0.50
2	F2	93±0.32
3	F3	98±0.41
4	F4	215±0.45
5	F5	277±0.43
6	F6	335±0.32
7	F7	116±0.34
8	F8	160±0.54
9	F9	225±0.36

All the values are expressed as a mean ± SD., n = 3

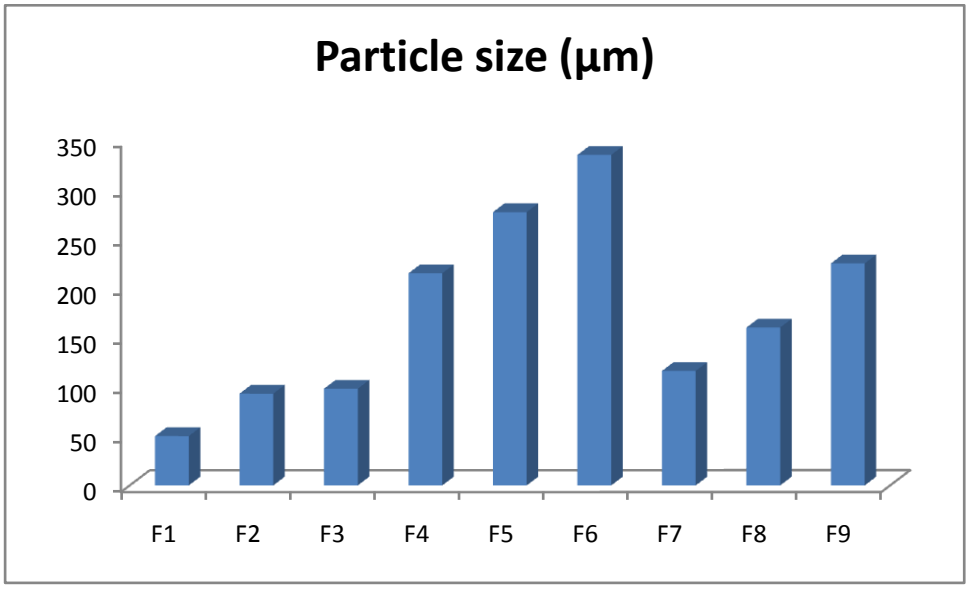


Figure 9.9: Mean Particles size

9.2.3. DRUG - POLYMERS COMPATIBILITY STUDIES:

9.2.3.1. Fourier Transform Infra-Red Spectroscopy (FTIR):

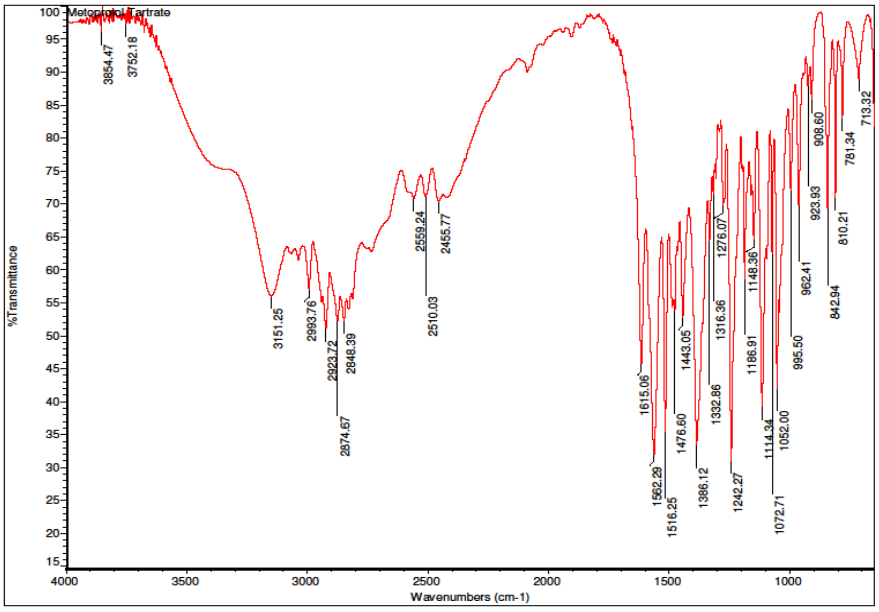


Fig 9.10: FTIR spectrum of pure drug Metoprolol Tartrate

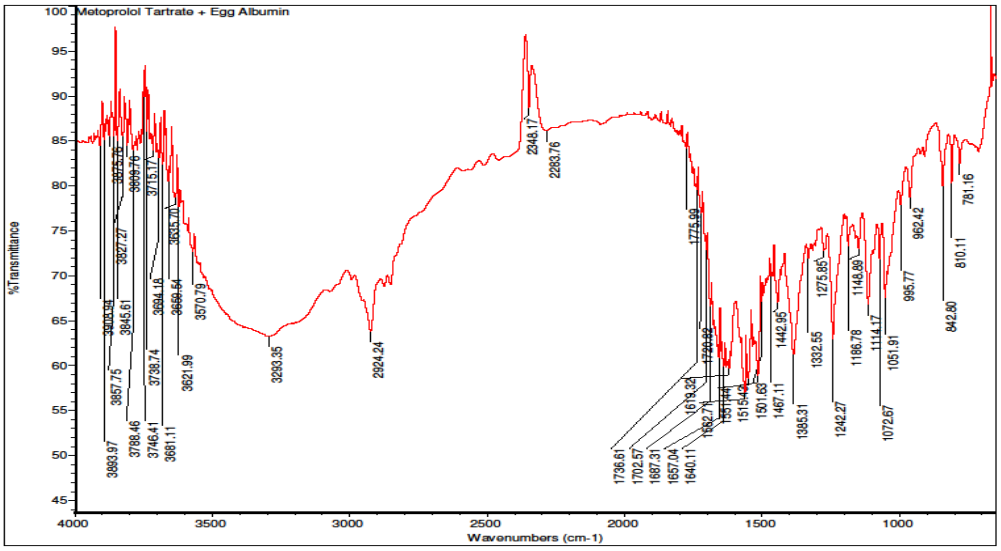


Fig 9.11: FTIR spectrum of Metoprolol Tartrate and Egg albumin

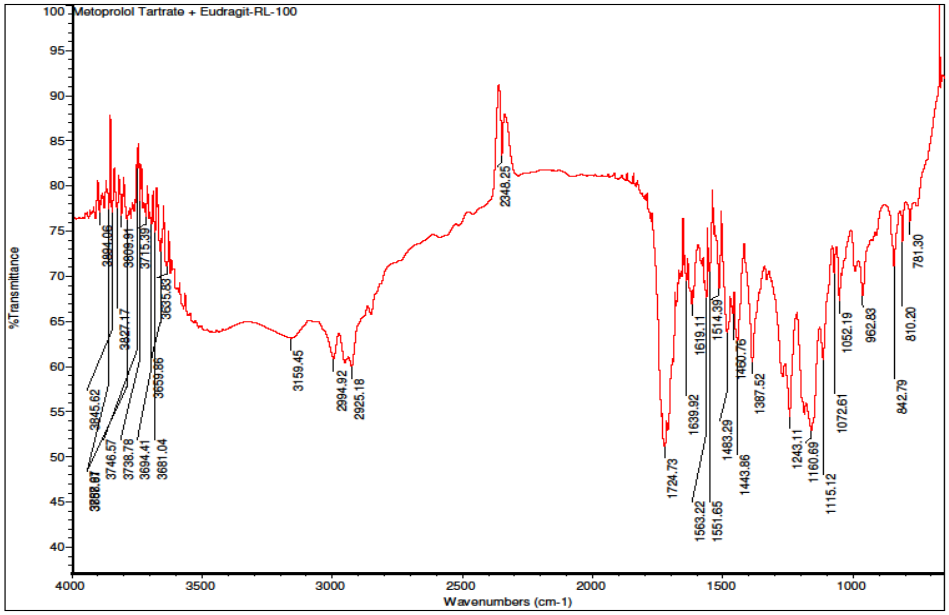


Fig 9.12: FTIR spectrum of Metoprolol Tartrate and Ethyl cellulose

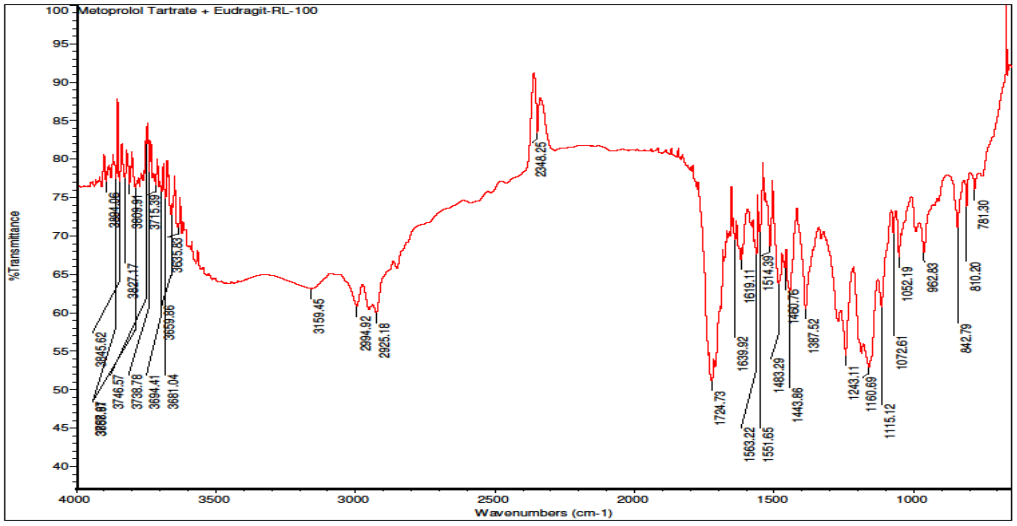


Fig 9.13: FTIR spectrum of Metoprolol tartrate and Eudragit RL 100

The possible drug and polymer interaction can be studied by FTIR spectroscopy. According to the Figure 9.10 and Figure 9.13 the major peaks observed in drug spectrum were also observed in spectrum of drug with polymer, therefore it could it could indicate that there is no in compatibility between drug and polymer**.**

9.2.3.2. Drug – polymer compatability

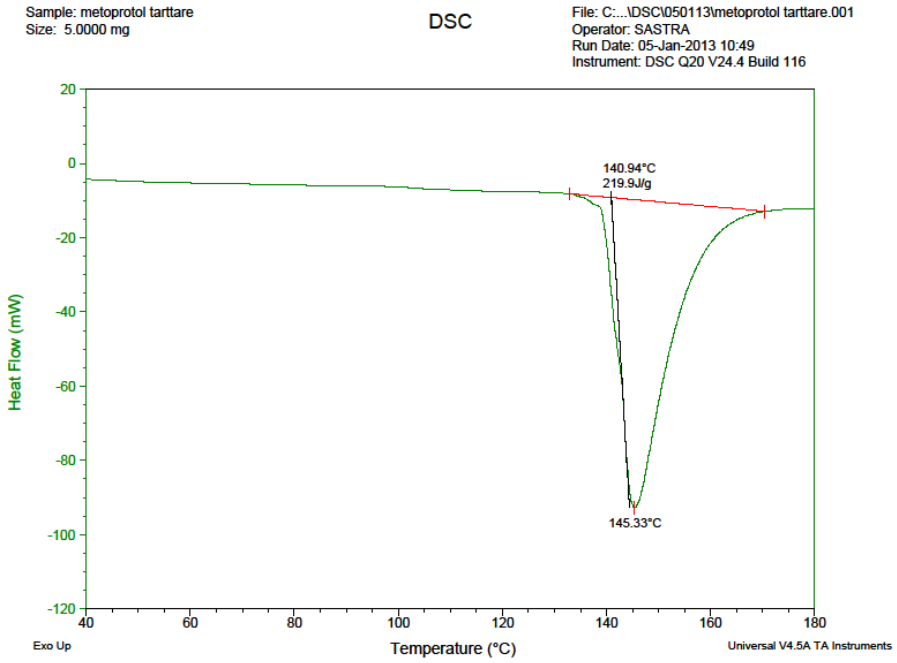


Fig.9.14: DSC Curve of Pure Metoprolol tartrate

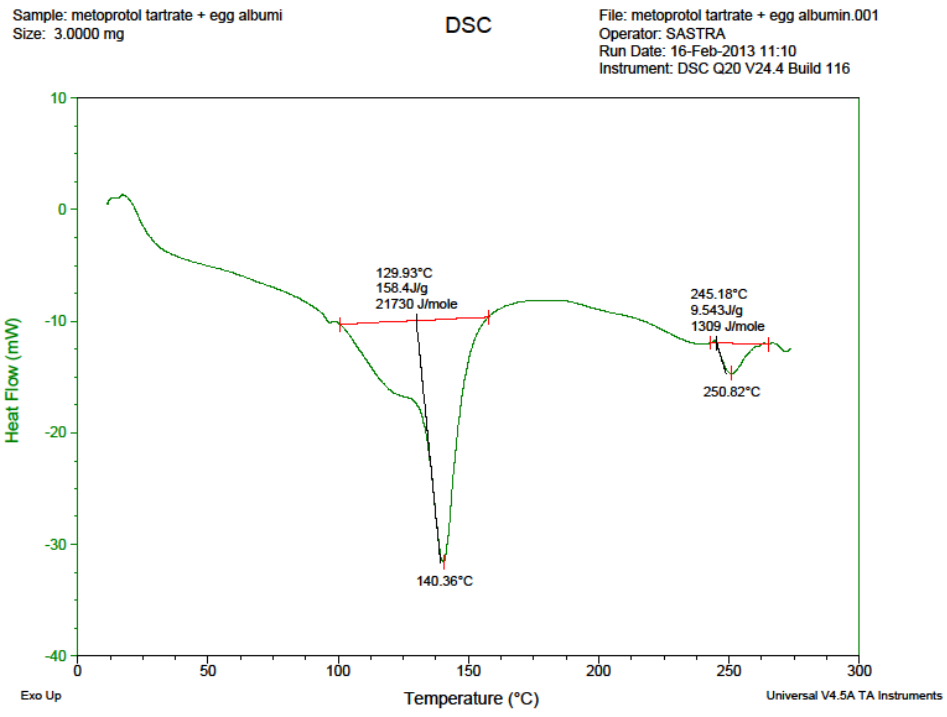


Fig.9.15: DSC Curve of Metoprolol tartrate and Egg albumin

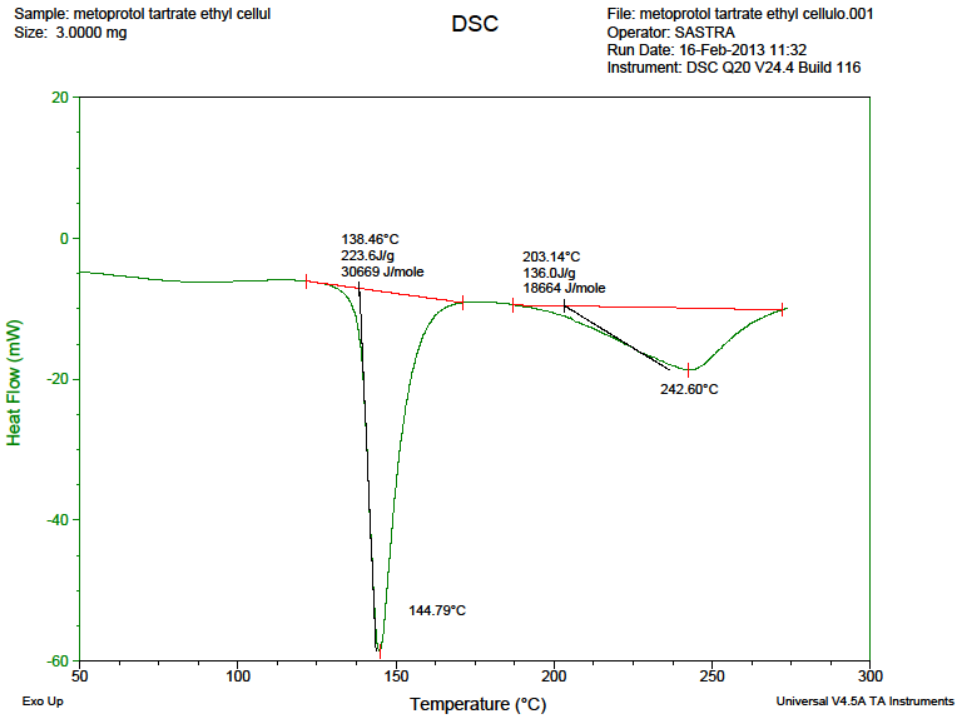


Fig.9.16: DSC Curve of Metoprolol tartrate and Ethyl cellulose

Table 9.13: Data for DSC thermogram parameters

S.NO	DSC thermogram sample	Onset temperature (°C)	Peak Temperature (°C)
1	**Metoprolol tartrate**	**140.94**	**145.33**
2	**Metoprolol tartrate +Egg albumin**	**129.93**	**140.36**
3	**Metoprolol tartrate +Ethyl cellulose**	**138.46**	**144.79**

9.2.4. Scanning Electron Microscopy Analysis (Surface morphology)

Surface morphology and shape characteristics of microspheres were evaluated by means of scanning electron microscopy. The SEM photographs of the microspheres revealed that the microspheres were spherical with rough, hollow surface and slightly aggregated were showed in Figure 9.17 and 9.18.

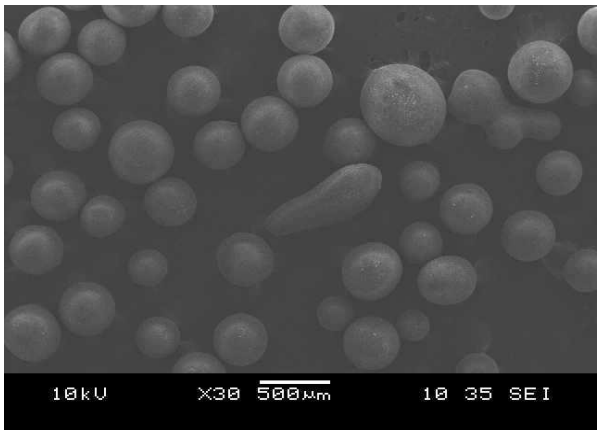


Figure 9.17: Scanning electron microphotograph of formulation F3 at lower Magnification

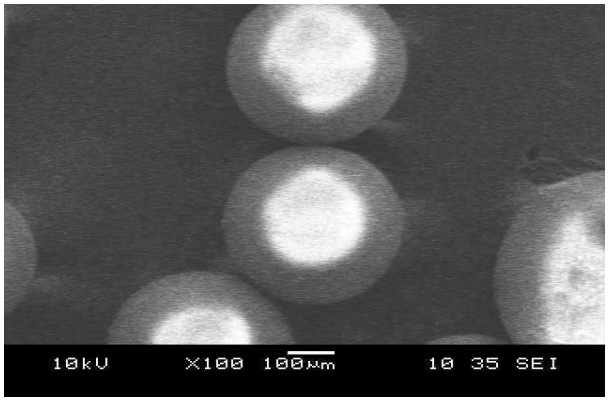


Figure 9.18: Scanning electron microphotograph of formulation F3 at higher Magnification

9.3. *In-vitro* Dissolution studies

In-vitro drug released profiles of Metoprolol tartrate microspheres were performed in each formulation up to 2 hours in 0.1N HCl followed by phosphate buffer (pH 7.4) up to 12 hours. It was represented in Table 9.24 and showed in Figure 9.19 to 9.27.

Table 9.14: Parameters were used for the dissolution study

Apparatus	USP type II apparatus (Basket type)
Temperature	37 ± 0.5° C
Total Volume	900ml
Speed	100 rpm
Drawn volume	5 ml
Running time	2 hrs in 0.1N HCl and 10 hrs in phosphate buffer pH 7.4.
Medium Replacement	Medium refilling at 2 hrs

Table 9.15: *In-vitro* drug release data of formulation F1

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
0	pH 1.2 (simulated gastric fluid)	0.00	0.00	0.00	0.00
0.5		11.46±0.70	11.46	5.94	0.25
1		20.22±1.35	20.22	11.30	0.47
2		26.31±1.42	26.31	17.99	0.71
3	pH 7.4 (simulated Intestinal fluid)	30.59±1.95	30.59	21.83	0.89
4		33.70±0.97	33.70	24.63	1.19
5		39.40±1.04	39.40	27.23	1.62
6		47.30±1.04	47.30	30.07	2.27
7		52.58±1.94	52.58	33.07	2.70
8		57.04±1.20	57.04	35.96	3.09
9		61.21±1.97	61.21	38.73	3.48
10		70.32±1.04	70.32	41.64	4.26
11		76.04±1.23	76.04	44.72	4.74
12		81.77±1.76	81.77	47.80	5.23

* All the values expressed as mean ± mean S.D., n=3

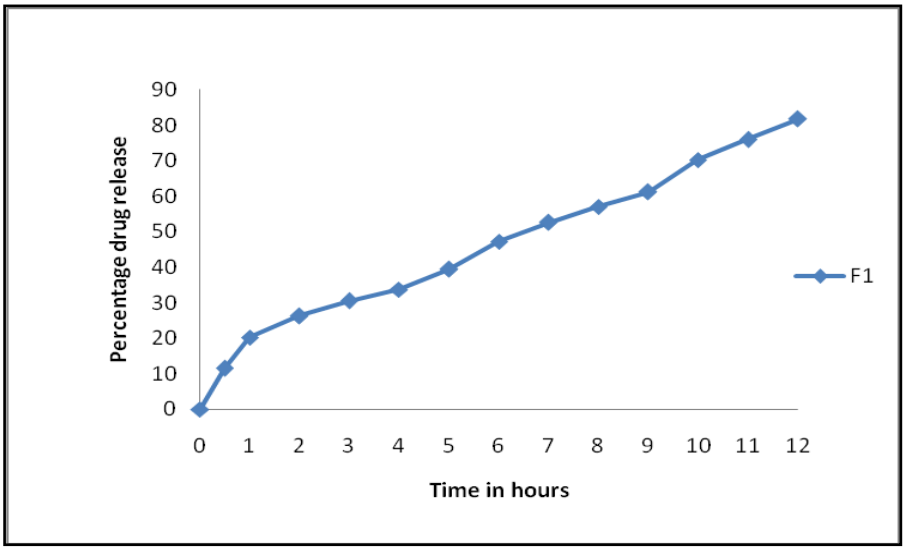


Fig. 9.19: *In-vitro* drug release profile of formulation F1

Table 9.16: *In-vitro* drug release data of formulation F2

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
0	pH 1.2 (simulated gastric fluid)	0.00	0.00	0.00	0.00
0.5		12.45±1.01	12.45	6.57	0.25
1		21.01±0.98	21.01	12.13	0.45
2		28.64±0.83	28.64	19.21	0.73
3	pH 7.4 (simulated Intestinal fluid)	31.96±1.54	31.96	23.27	0.85
4		36.43±1.95	36.43	26.15	1.18
5		42.27±1.76	42.27	28.94	1.64
6		48.33±1.20	48.33	31.82	2.13
7		52.57±1.89	52.57	34.64	2.50
8		56.47±1.27	56.47	37.31	2.86
9		61.50±1.29	61.50	39.92	3.34
10		73.18±1.29	73.18	42.87	4.32
11		79.07±0.69	79.07	46.11	4.80
12		83.60±1.24	83.60	49.29	5.17

* All the values expressed as mean ± mean S.D., n=3

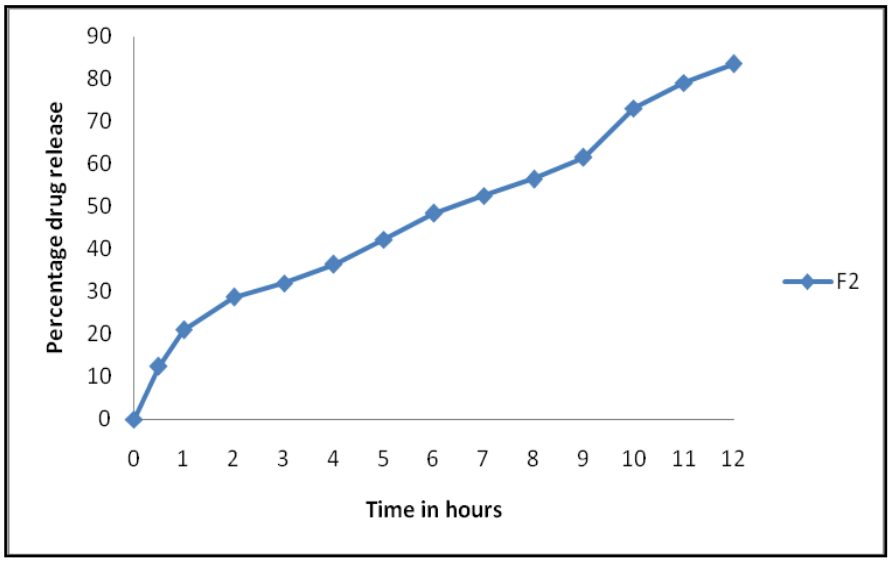


Fig 9.20: *In-vitro* drug release profile of formulation F2

Table 9.17: *In-vitro* drug release data of formulation F3

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
0	pH 1.2 (simulated gastric fluid)	0.00	0.00	0.00	0.00
0.5		14.27±1.61	14.27	7.60	0.25
1		28.92±0.98	28.92	15.24	0.50
2		32.39±0.68	32.39	23.87	0.61
3	pH 7.4 (simulated Intestinal fluid)	36.08±1.10	36.08	27.75	0.73
4		40.43±1.95	40.43	30.55	1.03
5		44.78±1.57	44.78	33.17	1.40
6		51.66±1.23	51.66	35.89	1.93
7		54.98±0.39	54.98	38.57	2.22
8		58.30±0.39	58.30	41.03	2.54
9		66.20±1.88	66.20	43.60	3.25
10		75.70±1.03	75.70	46.56	4.04
11		80.39±1.20	80.39	49.68	4.46
12		86.72±1.19	86.72	52.78	4.97

* All the values expressed as mean ± mean S.D., n=3

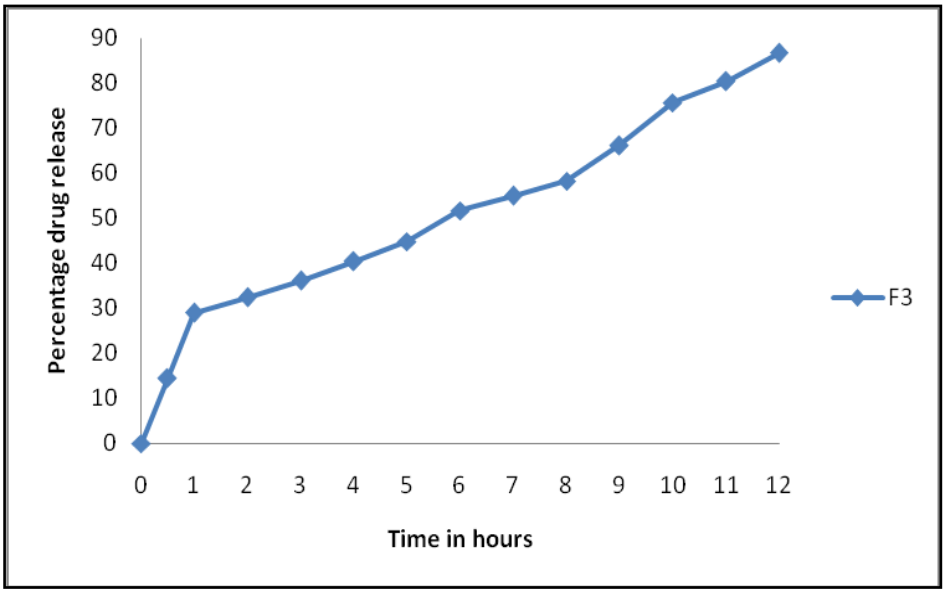


Fig 9.21: *In-vitro* drug release profile of formulation F3

Table 9.18: *In-vitro* drug release data of formulation F4

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
0	pH 1.2 (simulated gastric fluid)	0.00	0.00	0.00	0.00
0.5		8.81±0.82	8.81	4.68	0.25
1		11.52±0.68	11.52	7.75	0.37
2		18.03±0.79	18.03	11.77	0.78
3	pH 7.4 (simulated Intestinal fluid)	24.06±0.20	24.06	15.14	1.14
4		27.38±0.86	27.38	17.90	1.43
5		30.70±1.57	30.70	20.24	1.77
6		34.80±0.71	34.80	22.39	2.16
7		39.99±1.40	39.99	24.64	2.82
8		42.91±0.75	42.91	26.90	3.13
9		48.68±1.55	48.68	26.15	3.76
10		57.15±0.59	57.15	31.69	4.62
11		61.51±1.59	68.51	34.37	5.04
12		69.51±1.20	69.51	37.11	5.72

* All the values expressed as mean ± mean S.D., n=3

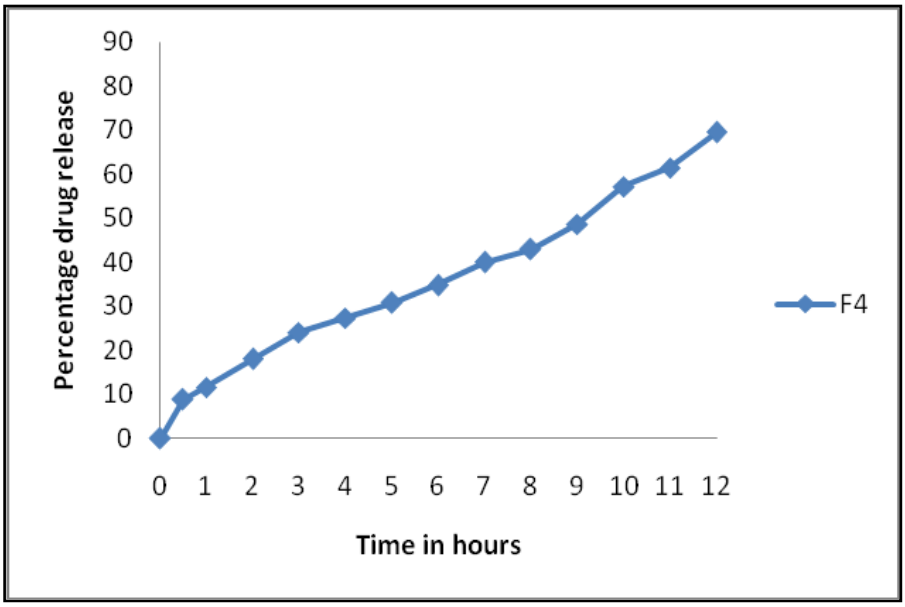


Fig 9.22: *In-vitro* drug release profile of formulation F4

Table 9.19: *In-vitro* drug release data of formulation F5

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
0	pH 1.2 (simulated gastric fluid)	0.00	0.00	0.00	0.00
0.5		10.11±0.69	10.11	19.76	0.25
1		16.53±0.86	16.53	21.62	0.37
2		18.93±1.30	18.93	22.06	0.40
3	pH 7.4 (simulated Intestinal fluid)	24.86±0.68	24.86	23.44	0.78
4		88.98±0.90	88.98	24.18	0.84
5		35.59±0.90	35.59	25.37	1.52
6		42.46±1.14	42.46	27.80	2.16
7		48.58±1.56	48.58	30.49	2.73
8		53.60±0.86	53.60	33.25	3.17
9		56.99±0.79	56.99	35.88	3.50
10		60.24±0.91	60.24	38.34	3.85
11		66.20±1.20	66.20	40.81	4.46
12		70.09±1.69	70.09	43.31	4.87

* All the values expressed as mean ± mean S.D., n=3

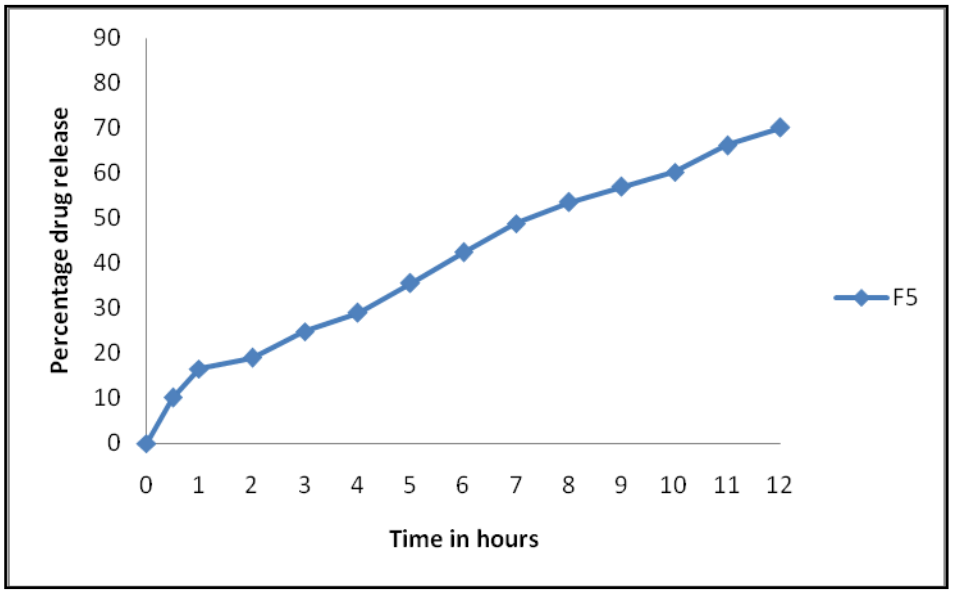


Fig 9.23: *In-vitro* drug release profile of formulation F5

Table 9.20: *In-vitro* drug release data of formulation F6

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
0	pH 1.2 (simulated gastric fluid)	0.00	0.00	0.00	0.00
0.5		10.98±1.17	10.98	5.83	0.25
1		19.35±0.49	19.35	10.95	0.47
2		21.96±1.31	21.96	16.45	0.59
3	pH 7.4 (simulated Intestinal fluid)	26.27±1.27	26.27	19.31	0.83
4		29.57±1.91	29.57	21.59	1.14
5		33.79±1.23	33.79	23.74	1.56
6		37.33±0.87	37.33	25.85	1.95
7		43.24±1.81	43.24	28.18	2.71
8		51.86±1.88	51.86	20.82	3.31
9		57.50±1.03	57.50	33.67	3.99
10		60.84±1.23	60.84	36.50	4.25
11		67.11±0.59	67.11	39.22	4.78
12		74.10±0.19	74.10	42.04	5.42

* All the values expressed as mean ± mean S.D., n=3

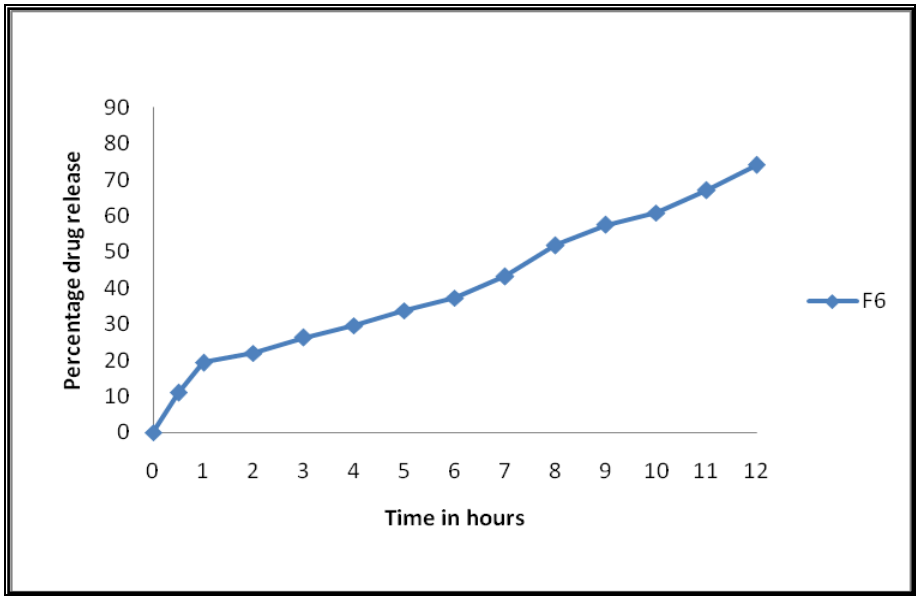


Fig 9.24: *In-vitro* drug release profile of formulation F6

Table 9.21: *In-vitro* drug release data of formulation F7

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
0	pH 1.2 (simulated gastric fluid)	0.00	0.00	0.00	0.00
0.5		10.87±1.67	10.87	5.77	0.25
1		16.63±1.04	16.63	10.18	0.42
2		19.35±0.99	19.35	14.66	0.58
3	pH 7.4 (simulated Intestinal fluid)	24.18±0.69	24.18	17.31	0.89
4		27.84±1.62	27.84	19.61	1.24
5		32.42±0.91	32.42	21.84	1.70
6		39.52±0.86	39.52	24.32	2.39
7		46.76±0.37	46.76	37.15	3.02
8		53.72±0.34	53.72	30.18	3.61
9		56.69±0.71	56.69	33.12	3.89
10		59.67±1.38	59.67	35.80	4.19
11		67.23±0.39	67.23	38.50	4.90
12		72.49±1.20	72.49	41.32	5.40

* All the values expressed as mean ± mean S.D., n=3

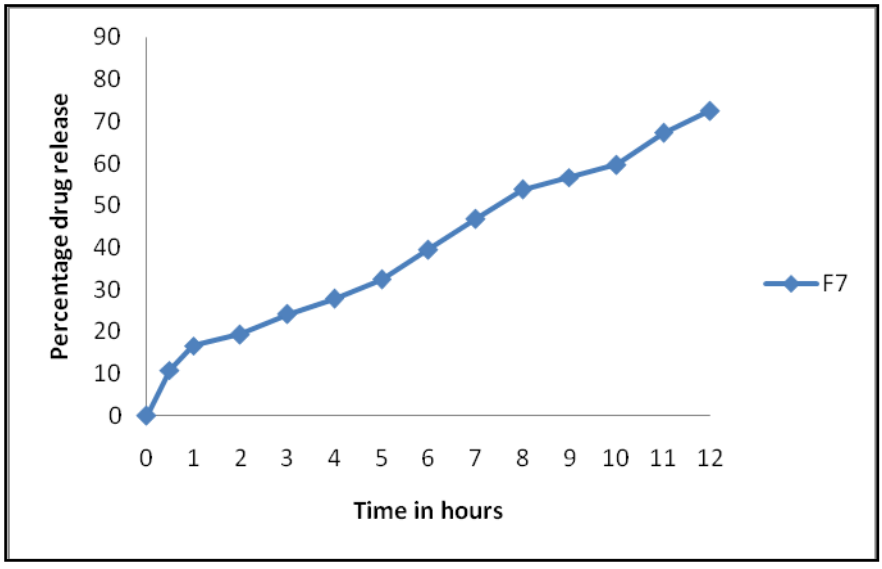


Fig 9.25: *In-vitro* drug release profile of formulation F7

Table 9.22: *In-vitro* drug release data of formulation F8

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
0	pH 1.2 (simulated gastric fluid)	0.00	0.00	0.00	0.00
0.5		11.63±1.30	11.63	6.17	0.25
1		18.15±0.86	18.15	10.98	0.27
2		21.09±0.98	21.09	31.85	0.43
3	pH 7.4 (simulated Intestinal fluid)	26.47±1.31	26.47	36.80	0.90
4		29.57±1.22	29.57	37.33	0.96
5		37.80±1.10	37.80	37.64	1.26
6		43.53±1.38	43.53	39.16	1.42
7		50.17±0.52	50.17	40.14	1.70
8		56.69±1.20	56.69	41.14	2.37
9		59.67±1.38	59.67	43.25	2.70
10		66.77±0.68	66.77	45.47	3.43
11		70.89±0.68	70.89	47.83	3.86
12		74.34±1.90	74.34	50.14	4.24

* All the values expressed as mean ± mean S.D., n=3

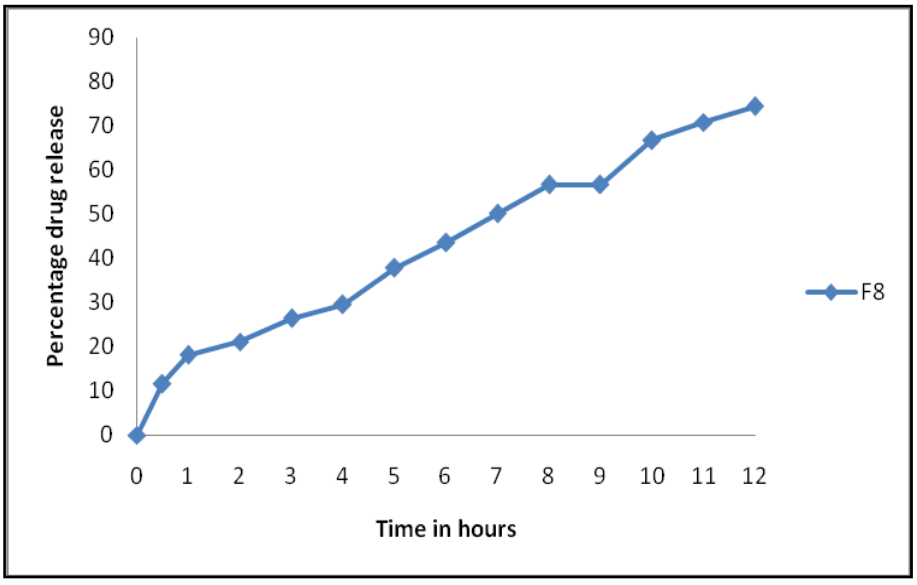


Fig 9.26: *In-vitro* drug release profile of formulation F8

Table 9.23: *In-vitro* drug release data of formulation F9

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
0	pH 1.2 (simulated gastric fluid)	0.00	0.00	0.00	0.00
0.5		12.29±1.63	12.29	6.51	0.25
1		19.24±0.99	19.24	11.61	0.43
2		24.46±1.31	24.46	17.41	0.66
3	pH 7.4 (simulated Intestinal fluid)	29.10±0.19	29.10	20.87	0.88
4		32.65±1.10	32.65	23.50	1.17
5		39.71±1.46	39.71	26.18	1.77
6		46.85±0.69	46.85	29.18	2.34
7		50.40±0.19	50.40	32.12	2.65
8		57.04±0.52	57.04	34.99	3.22
9		60.24±0.91	60.24	37.80	3.52
10		66.20±1.20	66.20	40.54	4.07
11		69.75±0.39	69.75	43.25	4.41
12		76.05±1.49	76.05	45.94	5.01

* All the values expressed as mean ± mean S.D., n=3

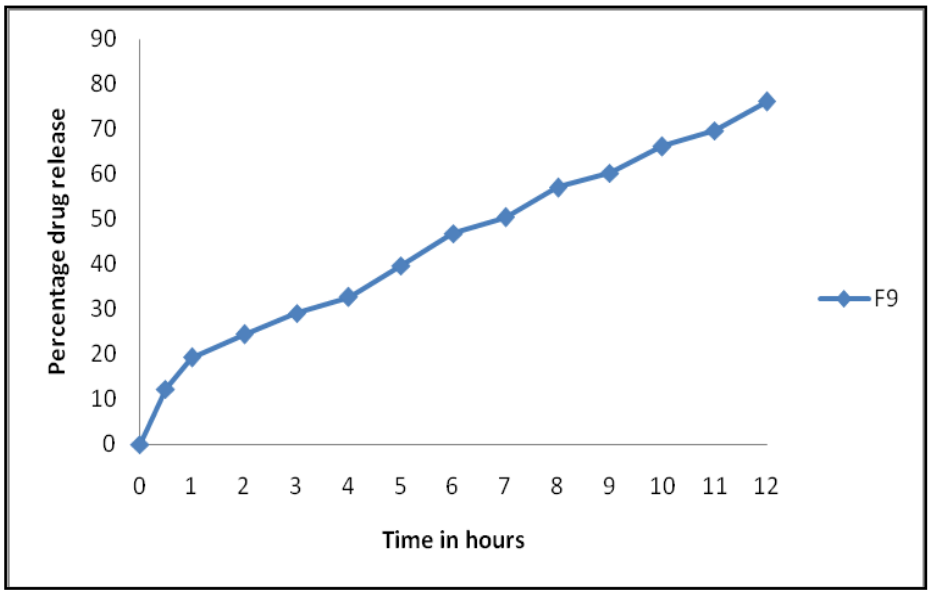


Fig 9.27: *In-vitro* drug release profile of formulation F9

Table 9.24: Comparative drug release data for all formulations

Time in hours	pH Medium	Formulations								
		F1 %	F2 %	F3 %	F4 %	F5 %	F6 %	F7 %	F8 %	F9 %
0.5	pH 1.2 (simulated gastric fluid)	11.46±0.70	12.45±1.01	14.27±1.61	8.81±0.82	10.11±0.69	10.98±1.17	10.87±1.67	11.63±1.30	12.29±1.63
1		20.22±1.35	21.01±0.98	28.92±0.98	11.52±0.68	16.53±0.86	19.35±0.49	16.63±1.04	18.15±0.86	19.24±0.99
2		26.31±1.42	28.64±0.83	32.39±0.68	18.03±0.79	18.93±1.30	21.96±1.31	19.35±0.99	21.09±0.98	24.46±1.31
3	pH 7.4 (simulated intestinal fluid)	30.59±1.95	31.96±1.54	36.08±1.10	24.06±0.20	24.86±0.68	26.27±1.27	24.18±0.69	26.47±1.31	29.10±0.19
4		33.70±0.97	36.43±1.95	40.43±1.95	27.38±0.86	88.98±0.90	29.57±1.91	27.84±1.62	29.57±1.22	32.65±1.10
5		39.40±1.04	42.27±1.76	44.78±1.57	30.70±1.57	35.59±0.90	33.79±1.23	32.42±0.91	37.80±1.10	39.71±1.46
6		47.30±1.04	48.33±1.20	51.66±1.23	34.80±0.71	42.46±1.14	37.33±0.87	39.52±0.86	43.53±1.38	46.85±0.69
7		52.58±1.94	52.57±1.89	54.98±0.39	39.99±1.40	48.58±1.56	43.24±1.81	46.76±0.37	50.17±0.52	50.40±0.19
8		57.04±1.20	56.47±1.27	58.30±0.39	42.91±0.75	53.60±0.86	51.86±1.88	53.72±0.34	56.69±1.20	57.04±0.52
9		61.21±1.97	61.50±1.29	66.20±1.88	48.68±1.55	56.99±0.79	57.50±1.03	56.69±0.71	59.67±1.38	60.24±0.91
10		70.32±1.04	73.18±1.29	75.70±1.03	57.15±0.59	60.24±0.91	60.84±1.23	59.67±1.38	66.77±0.68	66.20±1.20
11		76.04±1.23	79.07±0.69	80.39±1.20	61.51±1.59	66.20±1.20	67.11±0.59	67.23±0.39	70.89±0.68	69.75±0.39
12		81.77±1.76	83.60±1.24	86.72±1.19	69.51±1.20	70.09±1.69	74.10±0.19	72.49±1.20	74.34±1.90	76.05±1.49

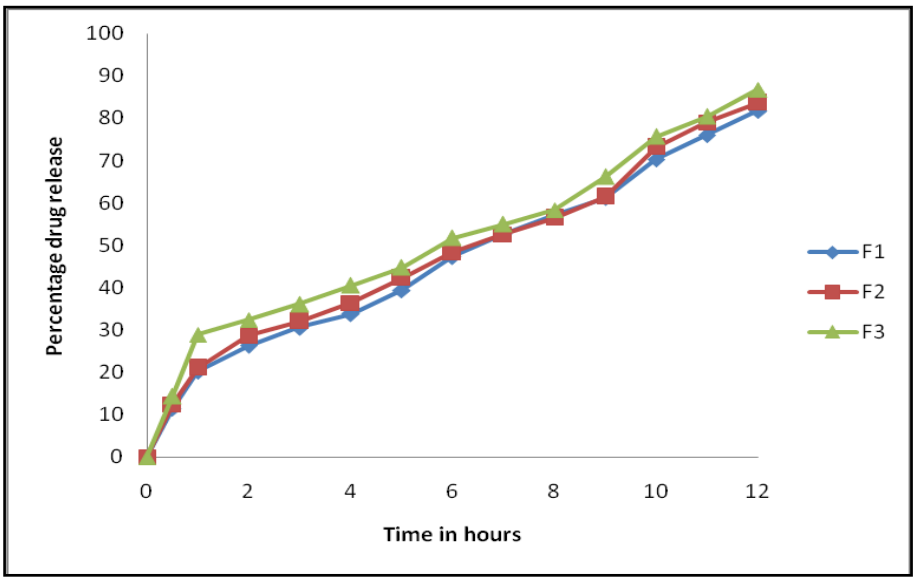


Fig.9.28: Comparitive drug release profile between F1 to F3

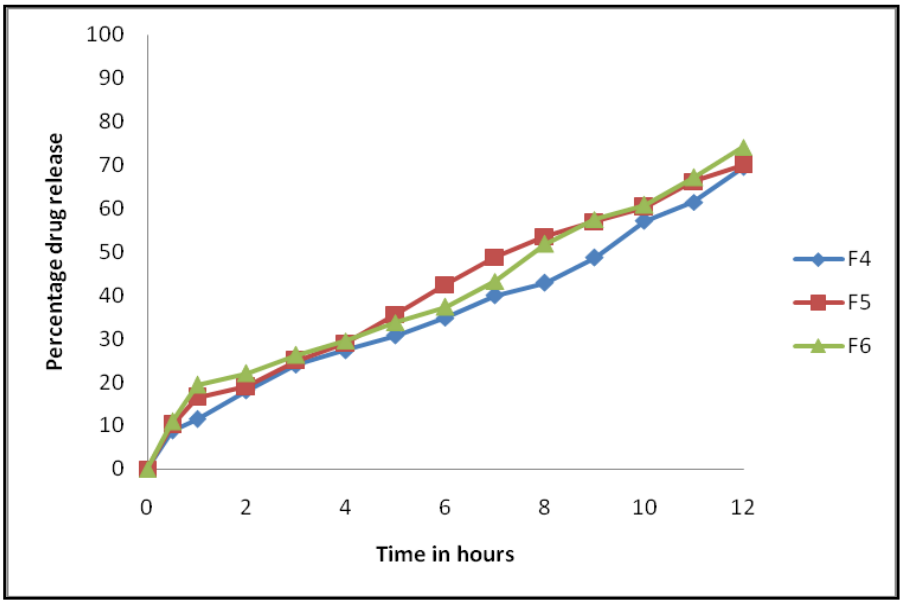


Fig.9.29: Comparitive drug release profile between F4 to F6

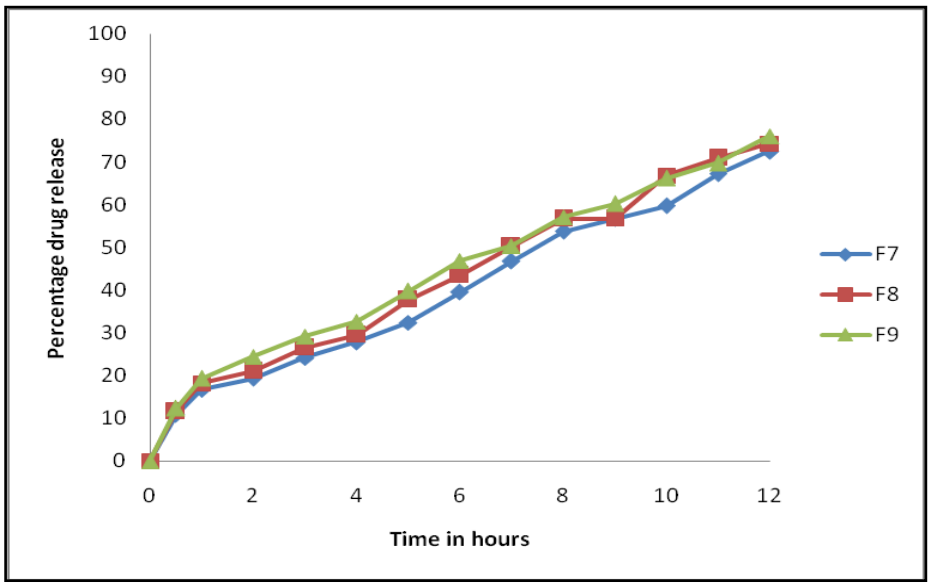


Fig.9.30: Comparitive drug release profile between F7 to F9

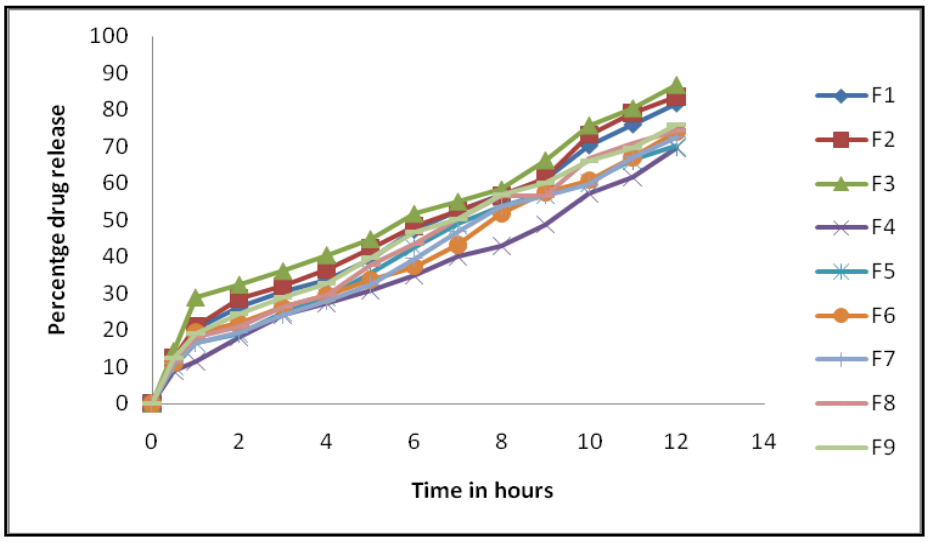


Fig 9.31: Comparative drug release profile between formulation F1 – F9

After completing the dissolution study in 0.1 N HCl (900ml) for first two hours and the dissolution study was continued in the phosphate buffer pH 7.4 up to the twelve hours. The drug release from formulation F1, F2 and F3 containing Egg albumin (1:1, 1:2 and 1:3) alone was found to be 81.77%, 83.60% and 86.72% % after the end of 12 hrs. This is due to higher solubility of drug in the medium.

The drug released from formulation F4, F5 and F6 containing Ethyl cellulose (1:1, 1:2 and 1:3) were 69.51%, 70.09% and 74.10% respectively at the end of 12 hrs.

The drug released from formulation F7, F8 and F9 containing Eudragit RL100 (1:1, 1:2 and1:3) were 72.49%, 74.34% and 76.05% respectively at the end of 12 hrs. The drug released from formulation F3containing Egg albumin was found to be 86.72% at the end of 12 hrs, which is showing high percentage drug release.

9.4. Kinetics of *In-Vitro* Drug Release:

The drug diffusion through most type of polymeric system is often best described by Fickian diffusion (diffusion exponent, n=0.5), but other process in addition to diffusion are important. There is also a relaxation of the polymer chain, which influences the drug release mechanism. This process is described as non-fickian or anomalous diffusion (n=0.5-1.0). Release from initially dry, hydrophilic glassy polymer that swell when added to water and become rubbery, show anomalous diffusion as a result of the rearrangement of macromolecular chain. The thermodynamics state of the polymer and penetrant concentration are responsible for the different type of the diffusion. A third class of diffusion is case-II diffusion (n=1), which is a special case of non- Fickian diffusion. To obtain kinetic parameter of dissolution profile, data were fitted to different kinetic models.

Table 9.25: Kinetics of *in-vitro* drug release profile for all Formulation

Formulation code	Zero orde R^2	First orde R^2	Higuchi's R^2 value	Peppas		Best fit
				R^2 value	n value	
F1	0.823	0.824	0.983	0.974	0.389	Matrix
F2	0.835	0.835	0.984	0.974	0.390	Matrix
F3	0.847	0.847	0.987	0.978	0.397	Matrix
F4	0.838	0.838	0.970	0.953	0.349	Matrix
F5	0.848	0.848	0.976	0.970	0.366	Matrix
F6	0.860	0.860	0.981	0.970	0.388	Matrix
F7	0.821	0.821	0.974	0.953	0.381	Matrix
F8	0.860	0.860	0.980	0.963	0.384	Matrix
F9	0.862	0.862	0.981	0.976	0.390	Matrix

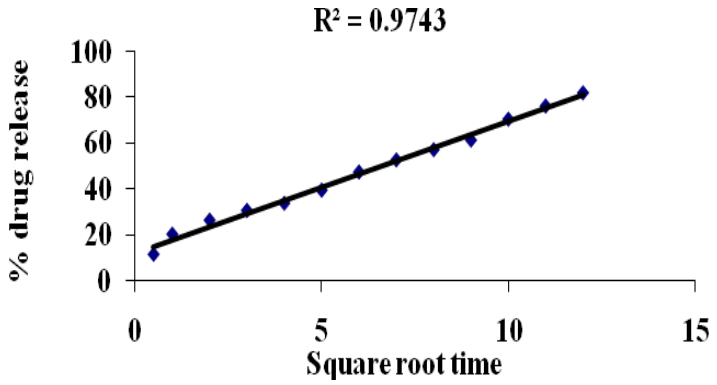


Fig. 9.32: Best fit model for formulation F1 (Matrix)

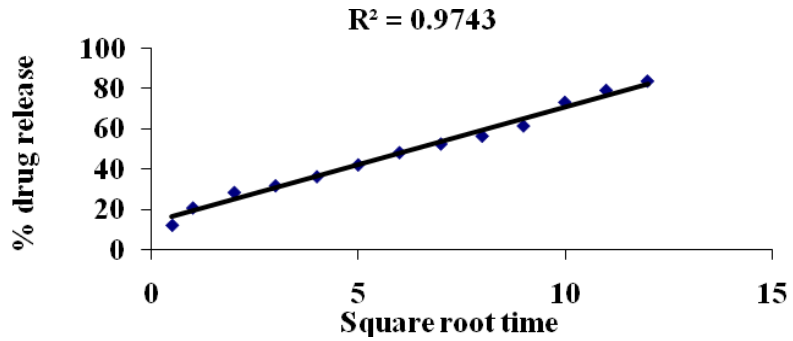


Fig. 9.33: Best fit model for formulation F2 (Matrix)

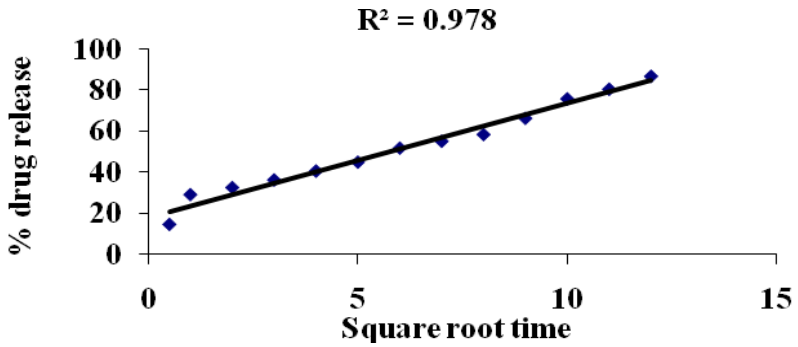


Fig. 9.34: Best fit model for formulation F3 (Matrix)

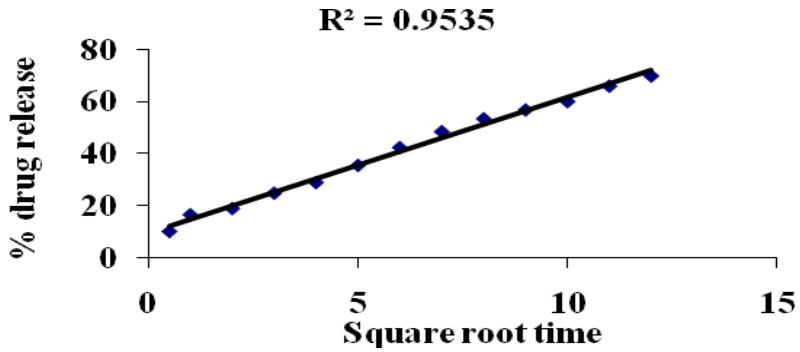


Fig. 9.35: Best fit model for formulation F4 (Matrix)

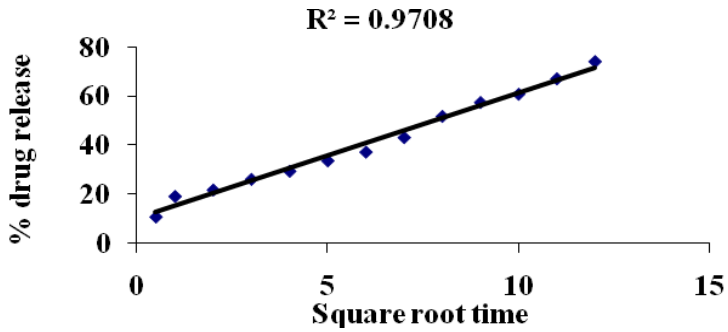


Fig. 9.36: Best fit model for formulation F5 (Matrix)

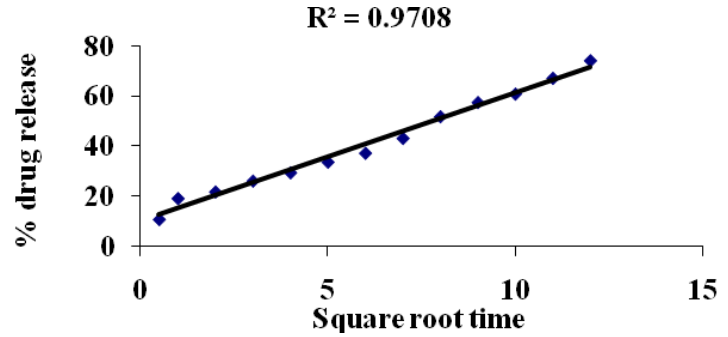


Fig. 9.37: Best fit model for formulation F6 (Matrix)

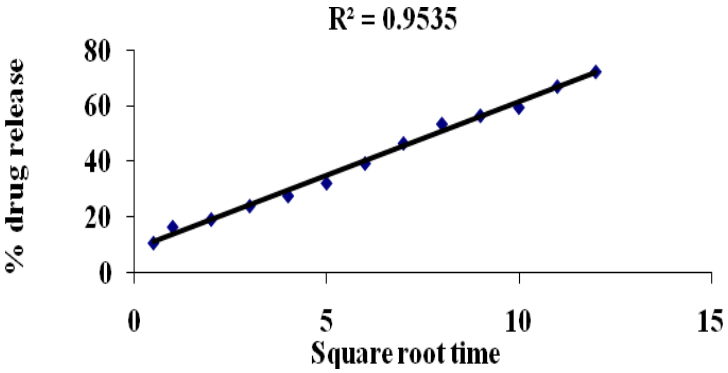


Fig. 9.38: Best fit model for formulation F7 (Matrix)

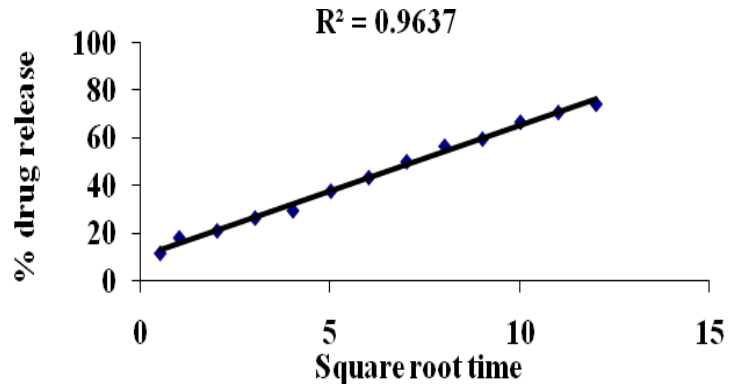


Fig. 9.39: Best fit model for formulation F8 (Matrix)

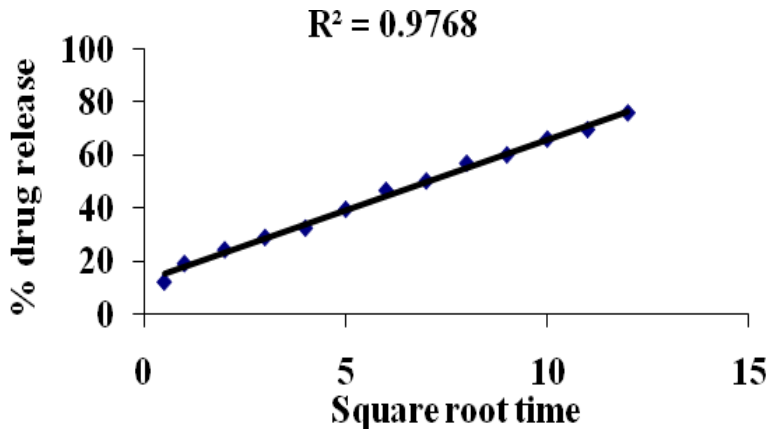


Fig. 9.40: Best fit model for formulation F9 (Matrix)

For microspheres, an "n" value near to 0.5 indicates diffusion control and an "n" value near to 1 indicates relaxation or erosion control. The intermediate value suggests that diffusion and erosion contributes to overall release mechanism. It was also observed that highest correlation was found for Matrix Square root time profile ($R^2 > 0.5$), which indicates the drug release via diffusion mechanism from all formulations.

9.5. STABILITY STUDY

From the results of the above studies it was found that formulation F3 was considered as the best formulation amongst the nine formulations. Hence formulation F3 was selected for stability studies.

9.5.1. Stability studies at the end of First month (30 days):

9.5.1.1. Drug content:

The Percentage drug content of microspheres after one month of stability studies was studied. The results are within the official limits. The data is shown in Table 9.26.

Table 9.26: Drug content of formulation F3 at the end of 1 month of stability

S. No.	Formulation	Percentage drug content
1.	F3	63.58±0.030

All the values are expressed as a mean ± SD., n = 3

9.5.1.2. Entrapment Efficiency:

The Entrapment efficiency of microspheres after one month of stability studies was studied. The results are within the official limits. The data is shown in Table 9.27.

Table 9.27: Entrapment efficiency of formulation F3 at the end of 1 month of stability

S. No.	Formulation	Entrapment efficiency (%)
1.	F3	88.37±0.030

All the values are expressed as a mean ± SD., n = 3

9.5.1.3. *In-vitro* drug release study:

The Percentage Drug Release from F3 microspheres after one month of stability was studied. The data is shown in Table 9.28

Table 9.28: *In-vitro* drug release data of formulation F3 at the end of 1 month of stability

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
0	pH 1.2 (simulated gastric fluid)	0.00	0.00	0.00	0.00
0.5		14.27±1.61	14.27	7.60	0.25
1		28.92±0.98	28.92	15.24	0.50
2		32.39±0.68	32.39	23.87	0.61
3	pH 7.4 (simulated Intestinal fluid)	36.08±1.10	36.08	27.75	0.73
4		40.43±1.95	40.43	30.55	1.03
5		44.78±1.57	44.78	33.17	1.40
6		51.66±1.23	51.66	35.89	1.93
7		54.98±0.39	54.98	38.57	2.22
8		58.30±0.39	58.30	41.03	2.54
9		66.20±1.88	66.20	43.60	3.25
10		75.70±1.03	75.70	46.56	4.04
11		80.39±1.20	80.39	49.68	4.46
12		86.72±1.19	86.72	52.78	4.97

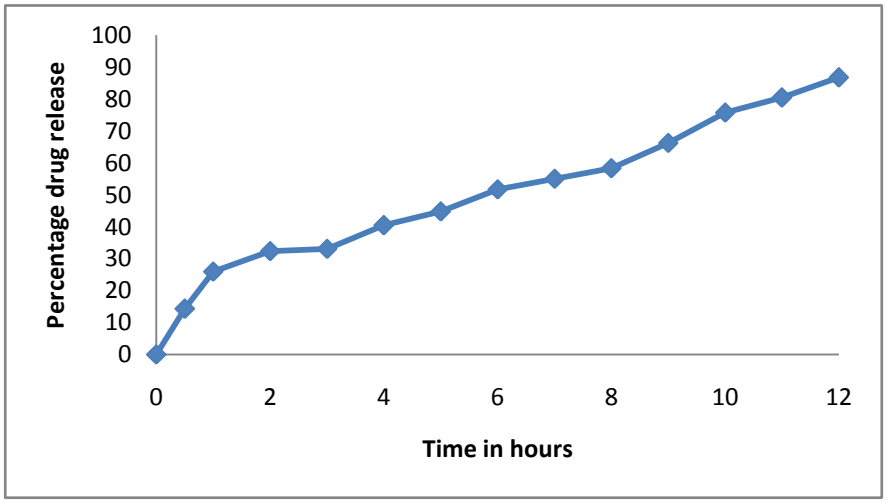


Fig.9.41: Stability study (*In-vitro* drug release) of formulation F3 at the end of 1 month

9.5.2. Stability studies at the end of Second month (60 days):

9.5.2.1. Drug content:

The Percentage drug content of microspheres after Two months of stability studies was studied. The results are within the official limits. The data is shown in Table 9.29.

Table 9.29: Drug content of formulation F3 at the end of 2 months of stability

S. No.	Formulation	Percentage drug content
1.	F3	63.54±0.025

All the values are expressed as a mean ± SD., n = 3

9.5.2.2. Entrapment efficiency:

The Entrapment efficiency of microspheres after Two months of stability studies was studied. The results are within the official limits. The data is shown in Table 9.30.

Table 9.30: Entrapment efficiency of formulation F3 at the end of 2 months of stability

S. No.	Formulation	Entrapment efficiency (%)
1.	F3	88.35±0.025

All the values are expressed as a mean ± SD., n = 3

9.5.2.3. *In-vitro* drug release study:

The Percentage Drug Release from F3 microspheres after Two months of stability was studied. The data is shown in Table 9.31.

Table 9.31: *In-vitro* drug release data of formulation F3 at the end of 2 months of stability

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
0	pH 1.2 (simulated gastric fluid)	0.00	0.00	0.00	0.00
0.5		14.27±1.61	14.27	7.60	0.25
1		28.92±0.98	28.92	15.24	0.50
2		32.39±0.68	32.39	23.87	0.61
3	pH 7.4 (simulated Intestinal fluid)	36.08±1.10	36.08	27.75	0.73
4		40.43±1.95	40.43	30.55	1.03
5		44.78±1.57	44.78	33.17	1.40
6		51.66±1.23	51.66	35.89	1.93
7		54.98±0.39	54.98	38.57	2.22
8		58.30±0.39	58.30	41.03	2.54
9		66.20±1.88	66.20	43.60	3.25
10		75.70±1.03	75.70	46.56	4.04
11		80.39±1.20	80.39	49.68	4.46
12		86.72±1.19	86.72	52.78	4.97

All the values are expressed as a mean ± SD., n = 3

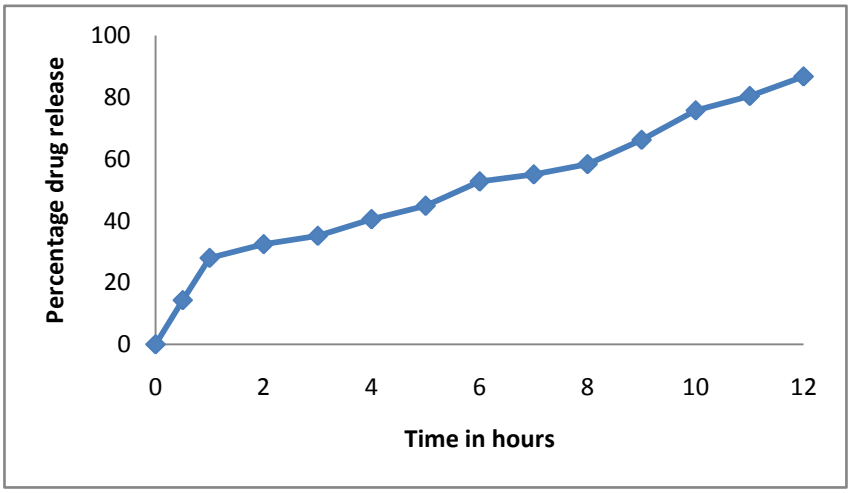


Fig.9.42 Stability study (*In-vitro* drug release) of formulation F3 at the end of 2 month

9.5.3. Stability studies at the end of Third month (90 days):

9.5.3.1. Drug content:

The Percentage drug content of microspheres after Third month of stability studies was studied. The results are within the official limits. The data is shown in Table 9.32.

Table 9.32: Drug content of formulation F3 at the end of 3 months of stability

S. No.	Formulation	Percentage drug content
1.	F3	63.51±0.020

All the values are expressed as a mean ± SD., n = 3

9.5.3.2. Entrapment efficiency:

The Entrapment efficiency of microspheres after Third month of stability studies was studied. The results are within the official limits. The data is shown in Table 9.33.

Table 9.33: Entrapment efficiency of formulation F3 at the end of 3 months of stability

S. No.	Formulation	Entrapment efficiency(%)
1.	F3	88.30±0.020

All the values are expressed as a mean ± SD., n = 3

9.5.3.3. *In-vitro* drug release study:

The Percentage Drug Release from F3 microspheres after Two months of stability was studied. The data is shown in Table 9.34.

Table 9.34: *In-vitro* drug release data of formulation F3 at the end of 3 months of stability

Time (hours)	pH Medium	DR* (%)	Amount (mg)	% DE	MDT
0	pH 1.2 (simulated gastric fluid)	0.00	0.00	0.00	0.00
0.5		12.45±1.01	12.45	6.57	0.25
1		21.01±0.98	21.01	12.13	0.45
2		28.64±0.83	28.64	19.21	0.73
3	pH 7.4 (simulated Intestinal fluid)	31.96±1.54	31.96	23.27	0.85
4		36.43±1.95	36.43	26.15	1.18
5		42.28±1.76	42.28	28.96	1.64
6		48.33±1.20	48.33	31.82	2.13
7		52.54±1.89	52.57	34.64	2.50
8		56.47±1.27	56.47	37.31	2.86
9		61.50±1.29	61.50	39.92	3.34
10		73.18±1.29	73.18	42.87	4.32
11		79.07±0.69	79.07	46.11	4.80
12		86.16±1.24	86.16	49.27	5.17

All the values are expressed as a mean ± SD., n = 3

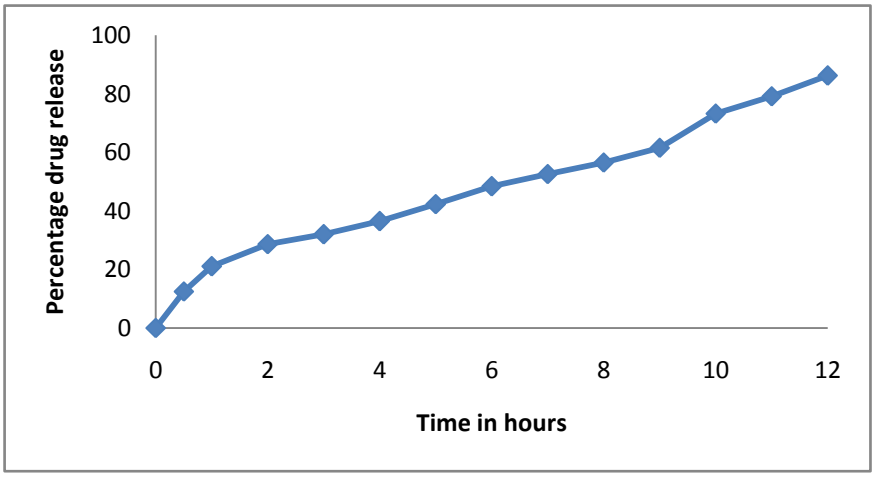


Fig.9.43. Stability study *(In-vitro* drug release) of formulation F3 at the end of 3month

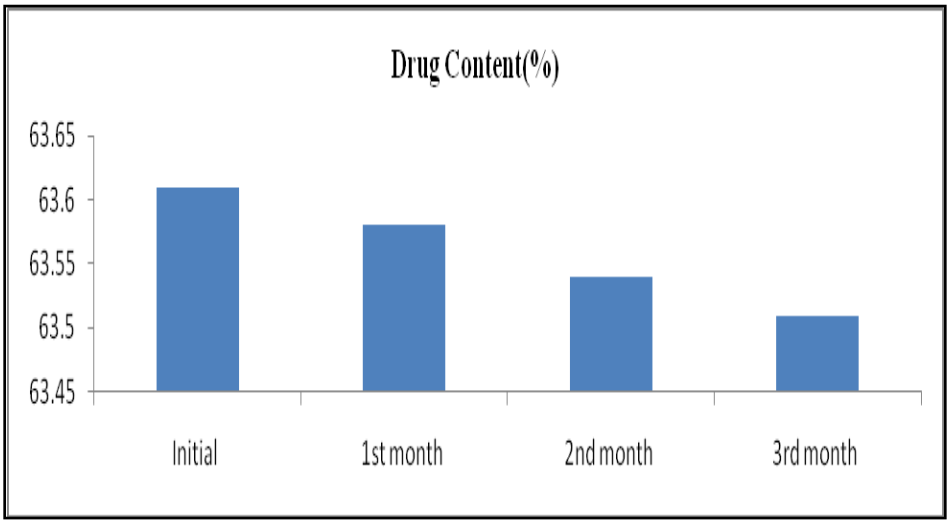


Fig.9.44: Comparison of drug content for formulation F3 with initial and different periods of stability

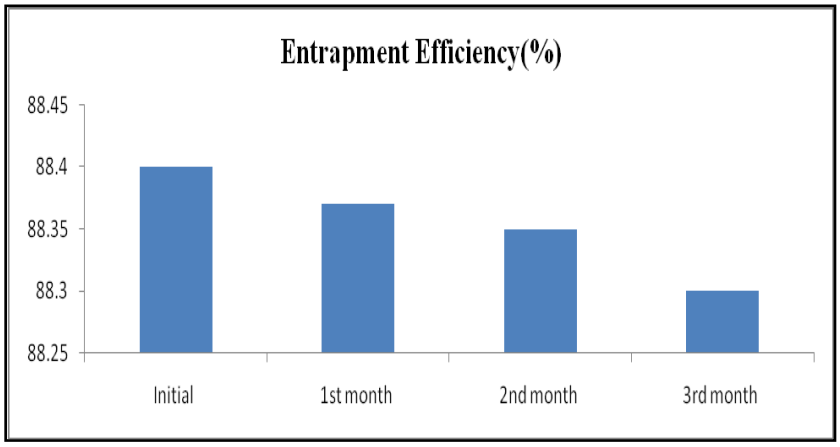


Fig.9.45: Comparison of Entrapment efficiency for formulation F3 with initial and different periods of stability

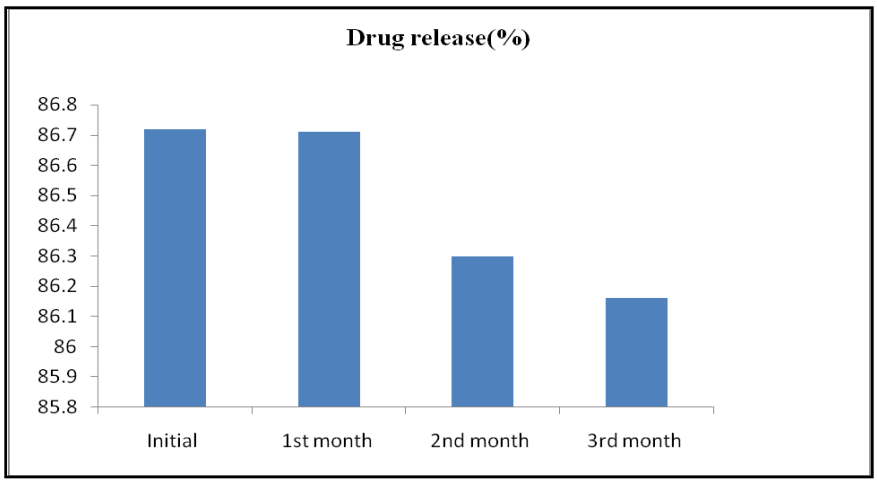


Fig.9.46: Comparison of percentage drug released at the end of 12 hours for formulation F3 with initial and different periods of stability

No statistically significant differences were observed in percentage drug content and percentage drug release in optimized formulation at the end of three months of stability studies. So it can be concluded that the formulation is stable for short term storage conditions.

SUMMARY &

CONCLUSION....

10. SUMMARY AND CONCLUSION

The goal of any drug delivery system was to provide the therapeutic amount of drug to the proper site in the body also to achieve and maintain the desired drug concentration in blood. Improving the therapeutic efficacy of existing drugs has been tried by different technologies. One of the effective technologies exiting in recent years of pharmacy is Microspheres.

Controlled release drug delivery system was developed in pharmacy field and drug retention for a prolonged time has been achieved. Hence, it was made an effective attempt to formulate the Controlled release microspheres by Metoprolol tartrate as the model drug.

Metoprolol tartrate was the prototype of cardio selective (β1) blocker used in the treatment of several diseases cardiovascular system especially hypertension. Metoprolol tartrate possess the mean half life of five hours and bioavailability was found to be only 30-40 % and high water solubility Hence, it was chosen as the good candidate for the Controlled release microspheres in order to improve the bioavailability and prolong period of drug released.

Controlled release microspheres of Metoprolol tartrate were successfully prepared by Emulsion polymerization and solvent evaporation technique was confirmed that it was a best method for preparing microspheres from its higher percentage yield.

The identification of drug was carried out by FTIR spectroscopy and melting point. The physicochemical parameters such as appearance, solubility study and loss on drying were performed by suitable methods. The analytical profile of drug was

evaluated for determination of absorption maximum, development of standard curve and percentage purity of drug.

Compatibility of drug and polymer mixture was done by performing DSC study. It was concluded that there was no interaction between the drug and polymer. Controlled release microspheres were obtained by solvent evaporation method for all the formulations from F1 to F9. Formulations F1, F2 and F3 were prepared Metoprolol tartrate with Egg albumin polymer in the ratio of 1: 1, 1: 2 and 1: 3. Similarly, formulations F4, F5 and F6 were prepared Metoprolol tartrate with Ethyl cellulose polymer in the ratio of 1:1, 1:2 and 1:3; and formulations F7, F8 and F9 were prepared Metoprolol tartrate with Eudragit RL 100 polymer in the ratio of 1: 1, 1: 2 and 1: 3. All formulations were evaluated for the Percentage yield, Drug content, Entrapment efficiency, Particle size, Scanning electron microscopy, and *In-vitro* drug release study.

On comparing the major criteria in evaluation such as percentage yield, drug content, entrapment efficiency and *In-Vitro* drug released profile, the **formulation F3** was selected as the best formulation, as it showed maximum percentage yield, drug content and Entrapment efficiency. It also showed a good Controlled drug release pattern up to 12 hrs.

Based on all the above evaluation parameters it was concluded that the formulation F3 was found to be best formulation among the formulations from F1 to F9. The *in-vitro* drug released data was applied to various kinetic models such as zero order kinetics, Higuchi plot, first order kinetics and Peppas plot by predict the drug release kinetics mechanism. The formulation F3 was best fitted with Matrix kinetics and it undergoes Quasi-fickian diffusion mechanism ($n<0.5$).

According to stability study it was found that there was no variation in Percentage yield, Entrapment efficiency, and *In-vitro* drug released profile of optimized formulation F3 for 3 months period.

From the overall studies it can be concluded that the formulation F3 considered as the best formulation among nine formulations by comparing all the evaluated parameters.

FUTURE PROSPECTS....

11. FUTURE PROSPECTS

In this work only physic-chemical characterization and *in-vitro* evaluation of Metoprolol tartrate were done.

- Along with in-vitro release study in-vivo release studies are also important. So in future in-vivo release study using different models are required to set the *in-vitro / in-vivo* correlation which is necessary for development of successful formulation and also long term stability studies are necessary.

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